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(21) International Application Number: PCT/US98/24950 (22) International Filing Date: 20 November 1998 (20.11.98) (30) Priority Data: 08/975,424 20 November 1997 (20.11.97) US (71) Applicants: GENETIX PHARMACEUTICALS, INC. [US/US]; 840 Memorial Drive, Cambridge, MA 02139 (US). MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US). (72) Inventors: LEBOULCH, Philippe; Flagship Warf, Unit 729, 197 8th Street, Charlestown, MA 02129 (US). PAWLIUK, Robert, James; Apartment 3, 52 Maple Avenue, Cambridge, MA 02129 (US). BACHELOT, Thomas; 53, rue Pierre Brunier, F-69300 Caluire (FR). (74) Agent: CLARK, Paul, T.; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110-2214 (US).			(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  Published With international search report.

(54) Title: ANTI-ANGIOGENIC GENE THERAPY VECTORS AND THEIR USE IN TREATING ANGIOGENESIS-RELATED DISEASES

(57) Abstract

A method for inhibiting tumor growth in a human patient harboring a solid tumor, said method comprising administering to said patient a nucleic acid molecule which expresses in said patient an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fragments thereof, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth.

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ANTI-ANGIOGENIC GENE THERAPY VECTORS AND  
THEIR USE IN TREATING ANGIOGENESIS-RELATED DISEASES

Field of the Invention

This invention relates generally to gene therapy for, e.g., cancer.

Background of the Invention

Angiogenesis is the process by which new capillaries are formed from existing vasculature. It is a complex process which involves proliferation and migration of endothelial cells. It plays a fundamental role in reproduction, development and wound repair. Unregulated angiogenesis, however, can further the progression of many diseases, including tumor growth and metastasis, arthritis, diabetes, and some forms of blindness. For example, there is experimental evidence that limits of tumor size and growth are not the failure of the tumor cells to proliferate, but rather a failure of the tumor to provide sufficient nutrients and waste removal to its constituent cells by recruiting surrounding vasculature.

Summary of the Invention

The invention features a method for inhibiting tumor growth in a human patient harboring a solid tumor, involving administering to the patient a nucleic acid molecule which expresses in the patient an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fragments thereof, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth.

In a second, related aspect, the invention features tumor inhibition, of

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the type just described, using nucleic acids molecules of the formula A-B.

where A and B are polypeptide and/or export signal joined by a peptide bond;

peptide A contains at least 100 amino acids and includes at least kringles 1, 2,

and 3 of human or murine angiostatin; and peptide B contains at least 100

5 amino acids and includes at least 75% of the amino acid sequence of human or murine endostatin. Expression of the fusion anti-angiogenic polypeptide in the

patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from

secreting transduced cells, thereby inhibiting its growth. In some embodiments

10 of this hybrid polypeptide and/or export signal method, polypeptide and/or

export signal A further includes kringle region 4 of angiostatin, and can also

include kringle region 5 of plasminogen (the larger protein molecule of which angiostatin is a portion).

In both aspects of the invention, the nucleic acid molecule preferably

15 constitutes a portion of a viral vector or a plasmid, which can either be

administered to the patient so that cells of the patient in the vicinity of the

tumor and/or systemically by diffusion of the recombinant protein to the

vascular compartment from secreting transduced cells are infected or

transfected with the nucleic acid encoding the angiogenesis-inhibiting

20 polypeptide, or cells (of the patient, or another human donor, or an animal) are

infected or transfected *ex-vivo*, and those infected or transfected cells are then

infused into the patient so that the anti-angiogenic polypeptide is expressed in

the vicinity of the tumor and/or systemically by diffusion of the recombinant

protein to the vascular compartment from secreting transduced cells.

25 As will be discussed in more detail below, in particularly effective embodiments, the nucleic acid molecule includes a nucleotide sequence

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encoding a preactivation polypeptide and/or export signal for effecting Golgi and/or endoplasmic reticulum export of the anti-angiogenic polypeptide.

In another aspect, the invention features a method for treating a human patient suffering from diabetic retinopathy, involving administering to  
5 the patient one of the nucleic acid molecules described above.

The above and other features, objects and advantages of the present invention will be better understood by a reading of the following specification in conjunction with the drawings.

#### Brief Description of the Drawings

10 Fig. 1 depicts the structural relationship of angiostatin with plasminogen.

Fig. 2 depicts the structural relationship of endostatin with collagen type XVIII.

15 Fig. 3 depicts various viral (A. MSCV murine retrovirus; B. Adeno-associated virus; C. HIV based retrovirus; E. recombinant adeno-virus) and non-viral (D. plasmid) vectors used in the construction of gene therapy vectors for this invention.

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MSCV: Murine Stem Cell Virus  
 LTR: Long Terminal Repeat  
 RSV: Rous Sarcoma Virus  
 ITR: Inverted Terminal Repeat  
 HIV: Human Immunodeficiency Virus  
 IRES: Internal Ribosomal Entry Site  
 GFP: Green Fluorescence Protein  
 HBPRE: Hepatitis B Export Element  
 RRE: Rev Response Element  
 polyA: polyadenylation site  
 $\Psi$ +: viral packaging sequence

The inverted triangle shows the site at which the anti-angiogenic constructs will be inserted using engineered MluI and XhoI restriction sites.

\* denotes specific mutations within the long terminal repeat and leader which bestows the ability for expression in embryonic stem and hematopoietic stem cells.

The arrow denotes the direction of transcription.

Fig. 4 depicts in the left (A) panel nude mice which were implanted with human neuroblastoma cells (line SK-N-AS) transduced with a mock virus and in the right (B) panel, nude mice which were transplanted with human neuroblastoma cells transduced with a retroviral gene therapy vector encoding an angiostatin-endostatin fusion protein.

Fig. 5 shows the nucleotide sequence (SEQ ID NO: 1) and amino

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acid sequence (SEQ ID NO: 2) of human plasminogen and the nucleotide sequence (SEQ ID NO: 5) and amino acid sequence (SEQ ID NO: 6) of human angiostatin.

Fig. 6 shows the nucleotide sequence (SEQ ID NO: 9) and amino acid sequence (SEQ ID NO: 10) of murine endostatin.

Fig. 7 shows the nucleotide sequence (SEQ ID NO: 3) and amino acid sequence (SEQ ID NO: 4) of murine plasminogen and the nucleotide (SEQ ID NO: 7) and amino acid sequence (SEQ ID NO: 8) of murine angiostatin.

#### Detailed Description

This invention provides gene therapy using a vector having a nucleotide sequence encoding one of the above-identified anti-angiogenic polypeptides. Described below in more detail are some of the components of the vectors and methods of the invention.

By a gene therapy vector is meant a vector useful for gene therapy. Gene therapy vectors carry a gene of interest that is useful for gene therapy. The gene therapy vectors are able to be transferred to the cells of an animal, e.g., a human, and are able to express the gene of interest in such cells so as to effect gene therapy. The vector can be, e.g., chromosomal, non-chromosomal, or synthetic, and can be RNA or DNA. The vector can be, e.g., a plasmid, a virus or a phage. Preferred vectors include, e.g., retroviral vectors, adenoviral vectors, adeno-associated vectors, herpes virus vectors, Simliki Forest Virus-based vector, Human Immunodeficiency virus, Simian Immunodeficiency virus, and non-viral plasmids. A preferred retroviral vector is Murine Stem Cell Virus (MSCV), which is a variant of Moloney Murine Leukemia Virus (MoMLV).

By anti-angiogenic polypeptide is meant a polypeptide which inhibits

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angiogenesis. The terms polypeptide, protein and polypeptide and/or export signal are used interchangeably herein. By angiogenesis is meant the process by which new vasculature, in particular, new capillaries, are formed from existing vasculature. Angiogenesis is a complex process entailing numerous steps, including local dissolution of the basement membrane, migration of endothelial cells into the surrounding stroma, proliferation of the endothelial cells at the leading edge to form a migrating column of cells, branching and fusion of the newly formed vascular loops, and formation of a new basement membrane. By inhibiting angiogenesis is meant completely or partially inhibiting the formation of such new vasculature.

In certain embodiments, the anti-angiogenic polypeptide is an anti-angiogenic fragment of plasminogen (in particular, angiostatin), an anti-angiogenic fragment of collagen XVIII (endostatin) or a fusion of the two fragments.

Angiostatin is an internal fragment of plasminogen having a molecular weight of 38 or 45 kDa, depending on whether it contains kringles 1-3 or 1-4. In the invention, either can be used, or a molecule including kringles 1-3 and a portion of kringle 4 can be used. Angiostatin can be naturally produced in vivo in small amounts by tumor cells, e.g. murine Lewis lung carcinoma cells, by proteolytic cleavage of plasminogen so as to eliminate the N-terminal portion including the signal polypeptide and/or export signal and the preactivation polypeptide and/or export signal, as well as the C-terminal portion following kringle 3 or 4. Mouse and human angiostatin have been purified and sequenced. In preferred embodiments, the gene therapy vectors of this invention encode angiostatin having kringles 1, 2 and 3, or angiostatin having kringles 1, 2, 3 and 4.

In another preferred embodiment, the anti-angiogenic polypeptide is



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endostatin or a biologically active analog or fragment thereof. Endostatin can be naturally produced *in vivo* in small amounts by tumor cells, e.g., murine angiosarcoma cells, by proteolytic cleavage of endogenous collagen XVIII so as to eliminate the N-terminal portion including the signal polypeptide and/or export signal and the preactivation polypeptide and/or export signal, as well as the C-terminal portion following kringle 3 or 4. See Fig.2. Mouse endostatin has been sequenced, and the human molecule (SEQ ID NOs: 17 and 18) forms a portion of collagen 18 (SEQ ID NOs: 19 and 20).

The human molecule position and sequence are apparent from an alignment of the active, Lys-terminated active region of human collagen 18 with murine endostatin, such that the C-terminal lysine residues align, bringing the active endostatin sequences into alignment.

In yet another preferred embodiment, the anti-angiogenic polypeptide is an in-frame fusion of angiostatin or a biologically active analog or fragment thereof and endostatin or a biologically active analog or fragment thereof. Preferably, the angiostatin or biologically active analog or fragment is 5' of the endostatin or biologically active analog or fragment. In certain embodiments, the angiostatin-endostatin fusion proteins exhibit synergistic anti-angiogenic properties.

By fragment is meant some portion of the naturally occurring anti-angiogenic polypeptide. Preferably, the fragment is at least 20 amino acid residues, more preferably at least 50 amino acid residues, and most preferably at least 100 amino acid residues in length. Fragments include chimeric constructs composed of at least a portion of the relevant gene and another molecule. The ability of a candidate fragment to exhibit a biological activity of the anti-angiogenic polypeptide can be assessed by methods known to those skilled in the art, e.g., by its ability to inhibit proliferation of bovine capillary

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cells, or by its ability to inhibit growth of primary tumor cells, e.g., as described herein. See, e.g., Example 9. Also included are fragments containing residues that are not required for biological activity of the fragment or that result from alternative mRNA splicing or alternative protein processing events.

5 Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide.

In preferred embodiments, the gene therapy vector of this invention is  
10 capable of hybridizing to the native anti-angiogenesis polypeptide-encoding regions and has at least about 80%, preferably at least about 90%, and more preferably at least about 95%, sequence identity to the native nucleotide sequences, and encodes a polypeptide which has anti-angiogenic activity; or a biologically active fragment of any of the above nucleotide sequences wherein  
15 the encoded polypeptide has anti-angiogenic activity.

The nucleotide sequences of the present invention can be in the form of RNA or DNA, and the nucleotide sequence can be double-stranded or single stranded and, if single stranded, can be the coding strand or non-coding (anti-sense) strand.

20 The coding sequence which encodes the anti-angiogenic polypeptide can be identical to the native coding sequences, or can be a different coding sequence which, as a result of the degeneracy of the genetic code, encodes the same anti-angiogenic polypeptide.

In certain embodiments, the gene therapy vector also has a nucleotide  
25 sequence encoding a signal polypeptide and/or export signal (SP) for effecting secretion of the anti-angiogenic polypeptide. Examples of signal polypeptide and/or export signal include plasminogen signal polypeptide and/or export

signal. Preferably, the signal polypeptide and/or export signal is 5' (i.e., upstream) of the nucleotide sequence encoding the anti-angiogenic polypeptide.

Preferably, the gene therapy vector has a nucleotide sequence encoding a preactivation polypeptide and/or export signal (PAP), which is a small polypeptide and/or export signal which effects folding and secretion of the anti-angiogenic polypeptide *in vivo*. Examples of preactivation polypeptide and/or export signal include plasminogen preactivation polypeptide and/or export signal, described herein, and PAP's of other proteins in the blood clotting cascade.

Preferably, the preactivation polypeptide and/or export signal is positioned 5' of the nucleotide sequence encoding the anti-angiogenic polypeptide. In embodiments which have a signal sequence and an anti-angiogenic polypeptide, preferably the preactivation polypeptide and/or export signal is 5' of the nucleotide sequence encoding the anti-angiogenic polypeptide, and 3' of the nucleotide sequence encoding the signal polypeptide and/or export signal.

We have discovered that results obtained using constructs containing a PAP- encoding nucleic acid sequence are far superior to results using constructs lacking a PAP-encoding sequence. Our hypothesis to explain these unexpectedly superior results with PAP is that, during the complex process by which the anti-angiogenic polypeptide is expressed and processed in living cells, the PAP polypeptide and/or export signal facilitates the export of the polypeptide from the cellular Golgi apparatus and/or the endoplasmic reticulum (ER). The corollary is that, absent PAP, a significant portion of the expressed polypeptide remains trapped in the Golgi and/or ER.

The PAP exemplified herein is derived from human plasminogen; this

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PAP is currently preferred. Our discovery that the use of a PAP dramatically improves results leads us to believe that other PAP's would be useful as well, and such others are therefore contemplated for use in the invention. Thus, as used herein, "PAP" refers to a polypeptide and/or export signal which is

5 naturally associated with a eukaryotic (preferably human) protein, the exportation of which is facilitated by its associated PAP. Examples of other human proteins whose Golgi/ER export is PAP-facilitated include other secreted proteins of the blood coagulation cascade, e.g., fibrinogen, prothrombin, Factor VIII, and Factor IX. Other secreted human proteins also

10 are associated with potentially useful PAPs.

It is not essential that the PAP used in the invention be identical in amino acid sequences to a native PAP; it is well-known that polypeptide and/or export signal that facilitate protein secretion or export, e.g., signal polypeptide and/or export signal and PAPs, can vary from the native forms to a certain

15 extent and still retain their function. Therefore, PAPs useful according to the invention preferably have 75% or greater amino acid sequence identity with a native PAP.

In certain embodiments, the gene therapy vector has a nucleotide sequence encoding a tag for identification of the anti-angiogenic polypeptide and/or export signal. In certain embodiments, the tag is 5' of the nucleotide

20 sequence encoding the anti-angiogenic polypeptide; in other embodiments, the tag is 3' of the nucleotide sequence encoding the anti-angiogenic polypeptide. In embodiments in which the anti-angiogenic polypeptide is endostatin or an angiostatin-endostatin fusion, it is preferred that the tag be 5' of the nucleotide

25 sequence encoding endostatin.

In certain embodiments the gene therapy vector includes a selectable

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marker, e.g., a Neomycin phosphotransferase gene, or a humanized red-shifted green fluorescent protein.

The invention also includes a cell infected or transfected with a gene therapy vector described herein. Preferably, the cell is an animal cell, more preferably an autologous or allogeneic human cell. The gene therapy vectors described herein can be introduced into a cell, e.g., by transformation, transfection, transduction, infection, or ex vivo injection. They can be targeted to a particular cell type.

Administration of nucleic acid, e.g., a gene therapy vector, can be accomplished by any method which allows the nucleic acid to reach the target cells. These methods include, e.g., injection, deposition, implantation, suppositories, oral ingestion, inhalation, topical administration, or any other method of administration where access to the target cells by the nucleic acid is achieved. Injections can be, e.g., intravenous, intradermal, subcutaneous, intramuscular or intraperitoneal. Implantation includes inserting implantable drug delivery systems, e.g., microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed, fused or partially fused pellets. Suppositories include glycerin suppositories. Oral ingestion doses can be enterically coated. Inhalation includes administering the nucleic acid with an aerosol in an inhalator, either alone or attached to a carrier that can be absorbed.

In certain embodiments of the invention, administration can be designed so as to result in sequential exposures to the nucleic acid over some time period, e.g., hours, days, weeks, months or years. This can be accomplished by repeated administrations of the nucleic acid, e.g., by one of the methods described above, or alternatively, by a controlled release delivery system in

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which the nucleic acid is delivered to the animal over a prolonged period without repeated administrations. By a controlled release delivery system is meant that total release of the nucleic acid does not occur immediately upon administration, but rather is delayed for some time. Release can occur in bursts  
5 or it can occur gradually and continuously. Administration of such a system can be, e.g., by long acting oral dosage forms, bolus injections, transdermal patches or subcutaneous implants. Examples of systems in which release occurs in bursts include, e.g., systems in which the nucleic acid is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being  
10 sensitive to a specific stimulus, e.g., temperature, pH, light, magnetic field, or a degrading enzyme, and systems in which the nucleic acid agent is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme. Examples of systems in which release of the nucleic acid is gradual and continuous include, e.g., erosional systems in which the nucleic acid is  
15 contained in a form within a matrix, and diffusional systems in which the nucleic acid permeates at a controlled rate, e.g., through a polymer. Such sustained release systems can be, e.g., in the form of pellets or capsules.

The nucleic acid is administered to the patient in a therapeutically effective amount. By therapeutically effective amount is meant that amount  
20 which is capable of at least partially preventing or reversing the disease. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the patient's size, age, the efficacy of the particular nucleic acid used, the type of delivery system used, the time of administration relative to the onset of disease symptoms, and  
25 whether a single, multiple, or controlled release dose regimen is employed. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

In certain embodiments, a therapeutically effective amount of an anti-angiogenic polypeptide is administered by providing to the animal a nucleic acid encoding the polypeptide and expressing the polypeptide in vivo. Nucleic acids encoding the polypeptide, or mutants thereof, can be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively delivering the nucleotide sequence for the anti-angiogenic polypeptide to cells in vivo. Approaches include, e.g., insertion of the nucleic acid into viral vectors. Viral vectors can be delivered to the cells, e.g., by infection or transduction using the virus. Viral vectors can also be delivered to the cells, e.g., by physical means, e.g., by electroporation, lipids, cationic lipids, liposomes, DNA gun,  $\text{Ca}_3(\text{PO}_4)_2$  precipitation, or delivery of naked DNA. In certain preferred embodiments, the virus is administered by injection, e.g., intramuscular injection, in a dose range of about  $10^3$  to about  $10^{10}$  infectious particles per injection, more preferably in a dose range of about  $10^5$  to about  $10^8$  infectious particles per injection. Single or multiple doses can be administered over a given period of time, depending, e.g., upon the disease.

An alternative is insertion of the nucleic acid encoding the anti-angiogenic polypeptide into a bacterial or eukaryotic plasmid. Plasmid DNA can be delivered to cells with the help of, e.g., cationic liposomes (lipofectin™; Life Technologies, Inc., Gaithersburg, MD) or derivatized (e.g., antibody conjugated) polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or  $\text{Ca}_3(\text{PO}_4)_2$  precipitation carried out in vivo, or by use of a gene gun. The above-described methods are known to those skilled in the art and can be performed without undue experimentation.

Since transfer of the nucleic acid to appropriate target cells represents the critical first step in gene therapy, choice of the particular gene delivery

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system will depend on such factors as the intended target and the route of administration, e.g., locally or systemically. Targets for delivery of the nucleic acid can be, e.g., specific target cells which are diseased. For example, the target can be, e.g., the peritoneal cavity, gastro-intestinal tract, bone marrow cavity, liver, lungs, muscles, vasculature, pericardial cavity, pleural cavity, skin, sub-cutaneous or deep connective tissues, central nervous system, spinal fluid, eye, or specific sites of tumor growth. Administration can be directed to one or more cell types, and to one or more cells within a cell type, so as to be therapeutically effective, by methods known to those skilled in the art. For example, the nucleic acid can be, e.g., coupled to an antibody, to a ligand to a cell surface receptor, or to a toxin component, or can be contained in a particle which is selectively internalized into cells, e.g., liposomes, or a virus where the viral receptor binds specifically to a certain cell type, or a viral particle lacking the viral nucleic acid, or can be administered by local injection.

In certain embodiments, the nucleic acid is administered to the patient by introducing *ex vivo* the nucleic acid into cells of the patient, or into syngeneic or allogeneic or xenogeneic cells, and then administering the cells having the nucleic acid to the animal. Any cell type can be used. In certain embodiments, the cells having the introduced nucleic acid are expanded and/or selected after the nucleic acid transfer. The cells having the transferred nucleic acid are subsequently administered to the patient. Preferably, the cells are administered in a dose range of about  $1 \times 10^6$  to about  $1 \times 10^9$  cells/dosage/day, and most preferably at about  $1 \times 10^7$  to about  $1 \times 10^8$  cells/dosage/day. The cells can be administered by any method which results in delivering the transferred nucleic acid in the cells to the desired target. For example, the cells can be implanted directly into a specific tissue of the patient, or implanted after encapsulation within an artificial polymer matrix. Examples of sites of



implantation include, e.g., the peritoneal cavity, gastro-intestinal tract, bone marrow cavity, liver, lungs, muscles, vasculature, pericardial cavity, pleural cavity, skin, sub-cutaneous or deep connective tissues, central nervous system, spinal fluid, eye, or specific sites of tumor growth.

5        Systemic delivery can be achieved, e.g., by introducing the nucleic acid into cells which circulate in the peripheral blood of the patient, or which give rise to cells which circulate in the peripheral blood. In certain embodiments, the nucleic acid is introduced into such cells *ex vivo*, and these cells are then administered to the patient, resulting in systemic delivery within the peripheral  
10    blood. These cells can be the cells of the patient or allogeneic cells. Preferred cells in which the nucleic acid can be introduced are hematopoietic cells.

      In certain embodiments, other therapy is additionally administered. For example, if the animal is being treated for a tumor, other tumor therapy, e.g., another therapeutic agent, chemotherapy, radiation or surgery, is additionally  
15    administered to the patient, either simultaneously or at different times.

      Treating is meant to include, e.g., preventing, treating, reducing the symptoms of, or curing the disease. I.e. treating a tumor includes preventing growth of the tumor, causing shrinkage of the tumor, or preventing development of micro-metastases.

20        Preferably, the recombinant nucleic acid is a gene therapy vector, e.g., as described herein. Preferably, the anti-angiogenic polypeptide is angiostatin, endostatin, an angiostatin-endostatin fusion protein, or biologically active analogs or fragments thereof. In certain embodiments, the angiostatin has kringles 1, 2 and 3; in other embodiments, the angiostatin has kringles 1, 2, 3  
25    and 4, and, in some embodiments, kringle 5 of human or murine plasminogen. Angiostatin is described in O'Reilly and Folkman U.S. Patent No. 5,639,725, hereby incorporated by reference. Endostatin is described in O'Reilly and

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Folkman PCT Appln. No. WO 97 15666, published May 1, 1997, hereby incorporated by reference.

In certain embodiments, the recombinant nucleic acid has been introduced *ex vivo* into cells so as to express the anti-angiogenic polypeptide in the cells, and the recombinant nucleic acid is administered to the patient by administering to the patient the cells containing the recombinant nucleic acid. In certain embodiments, the cells are derived from the patient; in other embodiments the cells are allogeneic cells relative to the cells of the patient.

Where cells are infected or transfected *ex vivo* for later infusion into the patient, the cells are preferably hematopoietic cells, but can also be mesenchymal cells, stem cells, epithelial cells (e.g., from the gut), or dendritic cells.

The gene therapy vectors of the invention can be provided in a pharmaceutical composition comprising a therapeutically effective amount of the recombinant nucleic acid together with a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include, e.g., water, saline, dextrose, glycerol, ethanol, liposomes and lipid emulsions.

The following non-limiting examples further illustrate the present invention.

#### EXAMPLES

##### Example 1: Construction of Inserts for Gene Therapy Vectors Containing cDNA for Angiostatin, Endostatin or Angiostatin-Endostatin Fusion Proteins

The following genetic constructs are inserted into retroviral gene therapy vectors; the genetic constructs contain human or murine cDNA for angiostatin, endostatin or an angiostatin-endostatin fusion, and DNA encoding a signal

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polypeptide and/or export signal (SP), a tag (FLAG), and, preferably, a preactivation polypeptide and/or export signal (PAP). The constructs are all made using standard genetic engineering techniques, and their insertion into retroviral gene therapy vectors is carried out using known methods. The

5 constructs have the following components:

Murine Constructs

SP-K1-K2-K3-Flag  
SP-K1-K2-K3-K4-Flag  
SP-K1-K2-K3-K4-K5-Flag  
10 SP-PAP-K1-K2-K3-Flag (SEQ ID NO: 11 and 12)  
SP-PAP-K1-K2-K3-K4-Flag (SEQ ID NO: 13 and 14)  
SP-Flag-Endo  
SP-K1-K2-K3-Flag-Endo  
SP-K1-K2-K3-K4-Flag-Endo (SEQ ID NO: 15 and 16)  
15 SP-PAP-K1-K2-K3-Flag-Endo

Human Constructs

SP-K1-K2-K3  
SP-K1-K2-K3-K4  
SP-K1-K2-K3-K4-K5  
20 SP-PAP-K1-K2-K3  
SP-PAP-K1-K2-K3-K4  
SP-PAP-K1-K2-K3-K4-K5  
SP-Endo  
SP-K1-K2-K3-Endo  
25 SP-PAP-K1-K2-K3-Endo

Nucleic acid and amino acid sequences for mouse and human angiostatin and endostatin used in these constructs are shown in Figs. 5-7.

Nucleic acid and amino acid sequence of the FLAG peptide:

amino terminus-	ASP	TYR	LYS	ASP	ASP	ASP	ASP	LYS
5'-	GAC	TAC	AAG	GAC	GAC	GAT	GAC	AAG

#### Human plasminogen derivative constructs

The entire coding region of the human plasminogen cDNA from the start (ATG) to the stop (TAA) codon is 2433bp in size.

This sequence encodes a signal peptide (bp 1-57), a preactivation peptide (bp 58-288), and 5 distinct structural regions known as kringles (K1-K3 from bp 289-1092; K4 from bp 1093-1380; K5 from bp 1381-1740). Please note that although I have given precise bp measurements for kringles K4 and K5, it can be argued that the sequence encoding K4 is between bp 1056-1440 and the sequence encoding K5 is between bp 1362-1680.

A DNA fragment encoding a portion of the human plasminogen protein from bp 1 to 1377 was obtained by PCR of a widely available human liver cDNA library using synthetic DNA oligonucleotides complementary to sequences immediately preceding the signal peptide and immediately following kringle 4. This fragment contains the signal peptide (bp 1-57), the preactivation peptide (bp 58-288), kringles 1 (bp 289-549), 2 (bp 550-804), 3 (bp 805-1092) and 4 (bp 1093-1380). The synthetic oligonucleotides used for this reaction contained engineered recognition sites for the restriction enzymes EcoRI and XhoI. Following the PCR reaction the amplified fragment was cloned into the EcoRI/XhoI sites of BluescriptSK(-) (Stratagene) using standard techniques (Maniatis). Following cloning the integrity of the amplified sequence was verified by sequencing both strands using the Sanger method (Sanger). Various derivatives of the cloned fragment were subsequently constructed using BluescriptSK(-) (Stratagene) as a backbone. A full list of the derivatives are described in Table 1. Briefly, the variations are composed of constructs containing various combinations of kringles with or without the signal and/or preactivation peptide sequences. These derivatives were constructed using both standard techniques as well as PCR and the use of double stranded synthetic oligonucleotides. In all cases the integrity of the start codon, coding sequence and termination codon was verified by double stranded sequencing using the Sanger method.

#### Murine plasminogen derivative constructs

The coding sequence for murine plasminogen is 2439bp in size and, similar to the human plasminogen cDNA encompasses a sequence encoding signal and preactivation peptides (bp 1-57 and 58-288 respectively) in addition to 5 kringle regions; kringle 1-3 (bp 289-1092), kringle 4 (bp 1093-1380) and kringle 5 (bp 1381-1743). Again, although I have given precise bp measurements for kringles K4 and K5, it can be argued that the sequence encoding K4 is between bp 1056-1440 and the sequence encoding K5 is between bp 1362-1680.

The murine plasminogen cDNA has previously been cloned and was made available to us. Derivatives of murine plasminogen were constructed using sequences derived from bp 1-1743 of the coding sequence. Various combinations of kringle regions with or without signal and preactivation peptide regions were made using BluescriptSK(-) (Stratagene, La Jolla, CA) as the vector backbone. These derivatives were constructed using standard cloning techniques (Maniatis, Molecular cloning; a laboratory manual, second edition, 1989) in combination with PCR utilizing synthetic oligonucleotides using

Angiostatin function was not altered by adding the FLAG polypeptide and/or export signal to either the N- or C-terminal ends, whereas endostatin was functional only if FLAG was added to its N-terminal end.

Example 2: Construction of Retroviral Gene Therapy Vectors

5 This example illustrates the construction of retroviral gene therapy vectors comprising cDNA for angiostatin, endostatin or angiostatin-endostatin fusion proteins.

The DNA inserts from Example 1 were inserted into two retroviral vectors. Both vectors were derived from the Murine Stem Cell Virus (MSCV),  
10 which is a variant of Moloney Murine Leukemia Virus (MoMLV) having several mutations allowing high, sustained expression in hematopoietic stem cells and their progeny. In both cases, the angiostatin, endostatin, or angiostatin-endostatin fusion DNA inserts were under the transcriptional control of the retroviral left Long Terminal Repeat (LTR). In the first vector,  
15 the dominant selectable marker was the Neomycin phosphotransferase gene (NeoR), which confers resistance to G418, and is driven by an internal phosphoglycerate kinase (PGK) promoter. In the second vector, the dominant selectable marker was the humanized, red-shifted green fluorescent protein (EGFP), which is co-translationally expressed by means of an Internal  
20 Ribosome Entry Site (IRES) from the Encephalomyocarditis virus (EMCV).

The retroviral gene therapy vectors were transfected by  $\text{CaPO}_4$  precipitation in the transient ecotropic packaging cell-line BOSC 23, Pear et al.,  
*PNAS* 90:8392 (1993). Viral supernatants were collected two days thereafter and filtered through 0.45 mm filters. Filtered viral supernatants were  
25 subsequently used to infect GENETIX's stable amphotropic retroviral packaging cell-line AM12 (Genetix Pharmaceuticals, Inc., Cambridge, MA). After another two days, viral supernatants from transduced AM12 were filtered

-20-

and used to infect GENETIX's stable ecotropic retroviral packaging cell-line GP+E86 (Genetix Pharmaceuticals, Inc.). Both transduced AM12 and GP-E86 were then selected in the presence of G418 (in the case of constructs bearing NeoR) or sorted by Fluorescent Activated Cell Sorter (FACS) for EGFP expression. Viral titers were estimated according to standard practice by counting G418 resistant colonies among NIH3T3 cells exposed to diluted virus preparation. Ecotropic viral titers were above  $5 \times 10^5$  /ml of viral supernatants, only 3-fold lower than "empty" control vectors. No Replication Competent Retrovirus (RCR) was detected in standard assays.

10 Example 3: Transduction of Target Cells Using Retroviral Gene Therapy Vectors

This example illustrates the stability of retroviral gene therapy vector transmission and the lack of toxicity in non-endothelial target cells.

Following 24-hour incubation of confluent viral producer cells in 100 mm plates, viral supernatant was removed and filtered (0.45  $\mu$ m filter, Gelman Sciences, Ann Arbor, MI). Viral supernatant, containing 7  $\mu$ g/ml polybrene (Sigma, St. Louis, MO), was added to target cells 24 hours after plating the target cells. Fresh medium was added after 4-12 hours, and, after an additional 48 hours, cells were selected for retroviral infection by exposure to medium containing 1 mg/ml G418 (Gibco BRL, Grand Island, NY) or by FACS sorting (FACStar cell sorter, Becton Dickinson, San Jose, CA). The stability of transmission of the retroviral gene therapy vectors described in Example 2 was examined by Southern blot analysis of transduced NIH3T3 cells, using specific probes (EGFP) and restriction enzyme digestion of genomic DNA with Sac I, which cuts only once in each LTR. Stable chromosomal integration of intact proviruses of appropriate length was observed with all constructs.

The lack of non-specific toxicity on non-endothelial cells was

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established by using filtered viral supernatants to transduce various tumor cell-types and cell-lines (NIH3T3 cells, K562 cells (ATCC), and human SK-N-AS neuroblastoma cells; Cohen, P.S., *Cancer Research*, 55:2380 (1995).

Transduced cell populations were subsequently selected with G418 or sorted for EGFP expression by FACS. No obvious effects on cell viability, growth or other phenotypical characteristics were detected.

Example 4: Protein Expression of Angiostatin, Endostatin and Angiostatin-Endostatin Fusion Proteins in Cells Transduced with Retroviral Gene Therapy Vectors

This example illustrates that recombinant angiostatin, endostatin, and angiostatin-endostatin fusion proteins were readily detected in retrovirally transduced cells and their supernatant, indicating efficient expression and secretion.

MSCV virus based vectors containing sequences encoding murine Kringle 1 (K1), K1K5, K1K2K3, K1K2K3K4, and K1K2K3K4K5 were used to transduce NIH3T3 cells. With regard to the murine recombinant proteins, Western blot analysis of transduced cells and their supernatant was performed by means of a monoclonal antibody that recognizes the FLAG polypeptide and/or export signal. Because this antibody is not mono-specific, significant cross-reactivity with murine proteins was apparent. However, by comparing the pattern obtained with mock cells, it was clear that the antibody revealed an additional band of appropriate size in all transduced cells. Moreover, the recombinant proteins were detected in cell supernatants at levels above 50 ng/ml, using a protein concentration/semi-purification procedure (Centricon columns, Amicon, Beverly, MA). With regard to the human recombinant proteins, no FLAG tag was added, so a monoclonal antibody that recognizes specifically the first three kringles of human plasminogen in its native, non-

-22-

denatured form was used; O'Reilly et al., *Cell* 79:315 (1994). Because of this constraint, Western blot analysis using denaturing gels could not be performed. An ELISA assay was performed which indicated that human recombinant angiostatin was detected at levels likely to be therapeutic according to previous findings in the model of Lewis Lung Carcinoma *Id*.

These results indicate that high levels of recombinant proteins of expected length were expressed in retrovirally transduced cells and were efficiently secreted.

10 Example 5: In Vivo Anti-Tumor Activity of Cells Transduced with Gene Therapy Vectors Encoding the Angiostatin-Endostatin Fusion Protein

Human SK-N-AS neuroblastoma cells (Cohen, 1995) were transduced with the retroviral gene therapy vector containing the angiostatin-endostatin fusion protein, described in Example 2. These cells (1,000,000) were suspended in 1 mL Dulbecco's phosphate buffered saline and injected into the right mid-quadrant of nude immuno-compromised mice. While no impairment of the *in vitro* growth of transduced cells was observed, a dramatic decrease in tumor growth in nude mice cells following subcutaneous implantation of the transduced cells was evident as compared to "mock virus"-transduced control cells.

20 Example 6: Ex Vivo Transfer of Retroviral Gene Therapy Vectors Encoding Anti-Angiogenic Polypeptides to Primary Hematopoietic Cells, and Subsequent Transplantation to Recipient Mice

This example illustrates infection of primary hematopoietic cells from



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donor mice with retroviral gene therapy vectors encoding angiostatin, endostatin, or an angiostatin-endostatin fusion protein, and a selectable GFP marker, and subsequent transplantation of the transduced hematopoietic cells into recipient mice.

5 Femoral bone marrow cells are harvested from male donor C57BL6/J-Ly5.1 mice (Jackson Labs, Bar Harbor, ME), intravenously injected four days previously with 150 mg/kg of 5-fluorouracil (5-FU). Bone marrow cells are cultured for two days in medium composed of DMEM, 15% fetal calf serum, 10 ng/ml human IL-6, 6 ng/ml murine IL-3 and 100 ng/ml murine Steel factor  
10 prior to two days of culture atop a confluent monolayer of irradiated (1,500 cGy,  $^{137}\text{Cs}$   $\gamma$ -irradiation) viral producer cells in the above medium including 6 ug/ml of prolamine sulfate. The viral producer cells are transfected with a retroviral gene therapy vector, as described above. Upon completion of the co-culture infection protocol, recovered non-adherent cells are cultured for an  
15 additional 48 hours to allow for expression of the transferred GFP gene. Retrovirally transduced cells expressing the transferred GFP gene are subsequently identified and selected for, using a FACStar+ cell sorter (Becton Dickinson, San Jose, CA). The GFP+ cells are intravenously injected into congenic female C57BL6/J-Ly5.2 recipient mice (National Cancer Institute,  
20 Washington, DC) previously given 950 cGy (83cGy/min,  $^{137}\text{Cs}$   $\gamma$ -rays) of whole body irradiation. In each case, a small fraction of GFP+ sorted cells is used for day 12 CFU-S and *in vitro* clonogenic progenitor assays to assess the efficiency of the infection and selection procedures on these more mature cell types.

25 Example 7: Engraftment of Recipient Mice with Donor-Derived Hematopoietic Cells

This example illustrates engraftment of the recipient mice with the

-24-

donor-derived transduced hematopoietic cells from Example 6.

The donor and recipient mice are phenotypically distinguishable on the basis of Y chromosome specific sequences, as well as on the basis of allelic differences at the murine CD45 cell surface antigen locus. Male donor mice are homozygous for the CD45.2 allele, while female recipient mice are homozygous for CD45.1. The engraftment of recipient mice with donor-derived (CD45.2+) cells is assessed at both short (5 weeks) and long (34 months) time points post-transplant by flow cytometric analysis of peripheral blood samples stained with a phycoerythrin labeled antibody specific for the CD45.2 antigen (Pharmingen, San Diego, CA). The results indicate that engraftment occurs.

Example 8: Proviral Marking and GFP Expression in Recipient Mice

This example illustrates the presence of recombinant provirus and expression of the transferred GFP gene in the recipient mice from Example 6.

The level of proviral marking in reconstituted animals is initially determined by Southern blot and semi-quantitative PCR analysis of DNA obtained from peripheral blood leukocytes. The large majority of donor-derived (CD45.2+) cells in recipient mice contain a minimum of one copy of recombinant provirus. In addition, flow cytometric analysis of peripheral blood leukocytes is performed to ascertain the proportion of cells expressing the transferred GFP cDNA. Because the GFP and angiogenic inhibitor protein cDNAs are both driven from the same regulatory sequences, due to the inclusion of an internal ribosomal entry site (IRES) element, the analysis of GFP expression in the peripheral blood provides an indirect measurement of the levels of anti-angiogenic protein being expressed. The results indicate expression of the transferred genes.

Example 9: Anti-Angiogenic Polypeptide Expression in Recipient Mice

This example illustrates the presence of anti-angiogenic polypeptide in the sera of the recipient mice from Example 6, using both physical and functional assays.

5 Serum obtained from the transplanted animals described in Example 6 is used for ELISA using an antibody specific for the synthetic FLAG epitope (IBI, Eastman Kodak, New Haven, CT) and compared against known standards of purified protein. Results indicate the presence of the anti-angiogenic polypeptide in the serum.

10 To determine whether a functional anti-angiogenesis polypeptide is present in the circulation, sera from transplanted animals is tested for its ability to inhibit the proliferation of bovine capillary cells *in vitro*; O'Reilly (1994). Briefly, cells are plated in 24 well dishes at 25,000 cells/ml and maintained in DMEM with 5% bovine calf serum for 24 hours. The medium is then replaced  
15 with fresh medium containing various dilutions of the test serum. After 20 minutes of incubation, fresh medium including b-FGF (final concentration 1 ng/ml) is added and the cells are cultured for 72 hours. Cells are then dispersed using trypsin and the cell number determined by Coulter counter. Results indicate that functional anti-angiogenic polypeptide is present in the sera of the  
20 recipient mice.

In addition, the ability of circulating anti-angiogenic polypeptide to inhibit the growth of primary tumor cells is assessed. Transplanted mice are subcutaneously injected with one million Lewis lung carcinoma (LLC) cells (O'Reilly, (1994)) at the proximal midline of their dorsal skin. The mice are  
25 closely monitored for survival, tumor size and growth, and overall health. Results indicate that the anti-angiogenic polypeptides from the sera of the

-26-

recipient mice inhibit growth of the LLC tumor cells.

Finally, upon sacrifice of the transplanted recipient mice, blood, spleen, thymus and bone marrow are harvested and analyzed for the presence of proviral DNA by Southern analysis as well as expression of the transferred GFP and anti-angiogenic polypeptide cDNAs by flow cytometry and ELISA. Moreover, a portion of bone marrow cells is re-transplanted into secondary recipients to generate individual day 12 spleen colonies, as well as plated in methylcellulose to assess *in vitro* clonogenic progenitors. Individual clones are analyzed for proviral DNA by PCR or Southern blot, and for gene expression by flow cytometry and ELISA. Results of these tests also indicate the presence of proviral DNA and expression of the anti-angiogenic polypeptides and marker proteins.

Example 10: Evaluating the Efficacy of Retroviral Gene Therapy Vectors Encoding Anti-Angiogenic Polypeptides on Various Human Cancers Implanted in SCID Mice Using Ex Vivo Gene Therapy

This example illustrates a method for rapidly screening various forms of human cancer to determine susceptibility to treatment by the systemic delivery of anti-angiogenic polypeptides.

The methods for gene transfer, assessment of proviral marking and assessment of transferred gene expression as described in Examples 3 through 9 are repeated using immuno-deficient SCID mice, with the following exceptions. Since SCID mice are more sensitive to  $\gamma$ -irradiation than C57BL6/J mice, the female SCID recipients receive a lower dose of 400cGy of whole body irradiation in contrast to the 950cGy required for C57BL6/J. In addition, since the SCID mice do not possess allelic differences at the CD45 cell surface antigen locus, donor and recipient cells are phenotypically distinguished on the basis of Y chromosome specific sequences using Southern

-27-

blot analysis.

Bone marrow from male donor SCID mice is infected, selected for on the basis of expression of the transferred GFP marker cDNA, and transplanted into irradiated female SCID recipients. Engraftment with provirally marked cells and expression of the transferred genes is demonstrated. The mice are then separately implanted with a variety of human tumor cell types, e.g., breast adenocarcinoma, lung squamous cell carcinoma, and brain glioblastoma. In each case, the ability of the anti-angiogenic polypeptides to inhibit the growth of the various human tumor cell types is monitored and quantified.

10 Example 11: Evaluating the Efficacy of Retroviral Gene Therapy Vectors Encoding Anti-Angiogenic Polypeptides for Treatment of Ovarian Cancer Using In Vivo Gene Therapy

This example illustrates the feasibility of using retroviral gene therapy vectors encoding anti-angiogenic polypeptides to achieve efficient gene transfer to established tumors in vivo using a well-established murine model of human ovarian cancer. Following injections, mice are closely monitored for tumor growth and survival.

Eight to ten week old nude mice (Jackson Labs, Bar Harbor) are injected intra-peritoneally with  $1 \times 10^7$  PA-1 cells, an ovarian cancer cell-line (ATCC), and followed until palpable tumors are identified. Viral supernatant for in vivo injection is prepared as follows: Viral producer cells are grown to confluence in DMEM with 10% bovine calf serum, and the medium is then changed. After 24 hours of incubation, the viral conditioned supernatant is filtered through a 0.45  $\mu$ m low protein binding filter, protamine sulfate is added to a final concentration of 6  $\mu$ g/ml. the solution is aliquoted into 2 ml volumes, and frozen at -80°C. Recipient mice receive three intraperitoneal injections of viral supernatant (2 mls per injection) in addition to the polycation, over a period of

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36 hours. Control mice are injected with medium collected from confluent dishes of NIH3T3 cells. Following injection of the viral conditioned supernatant, the mice are analyzed for survival as well as tumor growth over time as compared to mock injected controls. Results indicate that treatment of the ovarian cancer occurs. At death, the tumors are removed, weighed, and the cells dissociated for DNA extraction for Southern blot analysis to detect recombinant provirus.

Those skilled in the art will be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

In other embodiments, the invention provides methods and compositions for treating diseases and processes that are mediated by angiogenesis including, but not limited to, hemangioma, solid tumors, leukemia, metastasis, telangiectasia, psoriasis, scleroderma, pyogenic granuloma, myocardial angiogenesis, plaque neovascularization, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, corneal diseases, rubeosis, neovascular glaucoma, diabetic retinopathy, retrolental fibroplasia, arthritis, diabetic neovascularization, macular degeneration, wound healing, peptic ulcer, *Helicobacter* related diseases, fractures, keloids, vasculogenesis, hematopoiesis, ovulation, menstruation, placentation, and cat scratch fever.

What is claimed is:

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CLAIMS

1. Use of a nucleic acid molecule which expresses an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fragments thereof in the preparation of a medicament for inhibiting tumor growth in a human patient harboring a solid tumor, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth.

2. Use of a nucleic acid molecule which expresses an anti-angiogenic polypeptide of the formula A-B, wherein

A and B are polypeptide and/or export signal joined by a polypeptide and/or export signal bond;

A contains at least amino acids and comprises kringles 1, 2, and 3 of human or murine angiostatin; and

B contains at least amino acids and includes at least 75% of the amino acid sequence of human or murine endostatin in the preparation of a medicament for inhibiting tumor growth in a human patient harboring a solid tumor, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth.

3. The use of claim 2, wherein A further comprises kringle region 4 of

-30-

human or murine angiostatin.

4. The use of claim 2 or claim 3, wherein A further comprises kringle 5 of human or murine plasminogen.

5 5. The use of claim 1 or claim 2, wherein said nucleic acid molecule constitutes a portion of a viral vector.

6. The use of claim 1 or claim 2, wherein said nucleic acid molecule constitutes a portion of a plasmid.

10 7. The use of claim 6, wherein said plasmid is carried in a cell-free carrier so that the plasmid transfects living cells of the patient following plasmid administration, causing expression of the anti-angiogenesis polypeptide and/or export signal in the patient such that angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells is inhibited, causing inhibition of tumor growth.

15 8. The use of claim 6, wherein said plasmid has been transfected into animal cells *ex vivo*, wherein said animal cells express the anti-angiogenesis polypeptide to inhibit tumor-associated angiogenesis and tumor growth.

20 9. The use of claim 5, wherein said viral vector is carried in a cell-free carrier, so that the viral vector is incorporated into living cells of the patient following viral vector administration, causing expression of the anti-



-31-

angiogenesis polypeptide in the patient such that angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells is inhibited, causing inhibition of tumor growth.

5           10. The use of claim 5, wherein animal cells are infected with said viral vector *ex vivo* and then administered to said patient, wherein said animal cells express the anti-angiogenesis polypeptide to inhibit tumor-associated angiogenesis and tumor growth.

11. The use of claim 8, wherein said animal cells are human cells.

10           12. The use of claim 11, wherein said human cells are autologous.

13. The use of claim 11, wherein said human cells are allogeneic.

14. The use of claim 10, wherein said animal cells are human cells.

15. The use of claim 14, wherein said human cells are autologous.

16. The use of claim 14, wherein said human cells are allogeneic.

15           17. The use of claim 5, wherein said viral vector is a retroviral vector.

18. The use of claim 5, wherein said viral vector is a non-retroviral vector selected from the group consisting of adenoviral, adeno-associated, herpes, Simliki Forest virus, and poxvirus vectors.

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19. The use of claim 17, wherein said retroviral vector is Murine Stem Cell Virus or a lentivirus.

20. The use of claim 1, wherein said angiostatin comprises kringles 1, 2 and 3.

5        21. The use of claim 20, wherein said angiostatin further comprises kringle 4.

22. The use of claim 1, wherein said anti-angiogenic polypeptide is a fusion of angiostatin or a biologically active fragment thereof and endostatin or a biologically active fragment thereof.

10       23. The use of claim 1, wherein said nucleic acid molecule includes a nucleotide sequence encoding a signal polypeptide and/or export signal for effecting secretion of said anti-angiogenesis polypeptide.

24. The use of claim 23, wherein said signal polypeptide and/or export signal is plasminogen signal polypeptide and/or export signal.

15       25. The use of claim 1, wherein said nucleic acid molecule includes a nucleotide sequence encoding a preactivation polypeptide and/or export signal for effecting Golgi and/or ER export of the anti-angiogenic polypeptide..

26. The use of claim 25, wherein said preactivation polypeptide and/or export signal is a preactivation polypeptide and/or export signal of a human  
20    protein of the blood coagulation cascade.

27. The use of claim 26, wherein said preactivation polypeptide and/or export signal is human plasminogen preactivation polypeptide and/or export signal.

28. The method of claim 25, wherein the preactivation encoding  
5 sequence is positioned between a signal-encoding sequence and the sequence encoding the anti-angiogenic polypeptide and/or export signal.

29. The use of claim 1, wherein said nucleic acid molecule includes a nucleotide sequence encoding a tag for identification of said anti-angiogenic polypeptide.

10 30. The method of claim 27, wherein said tag is a Flag tag polypeptide and/or export signal.

31. A viral gene therapy vector comprising a nucleic acid molecule which encodes an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine  
15 endostatin, and angiogenesis-inhibiting fusions and fragments thereof, wherein said viral vector is sufficiently attenuated for use in human gene therapy.

32. A human cell infected with the vector of claim 31.

33. Use of a nucleic acid molecule which expresses in said patient an anti-angiogenic polypeptide selected from the group consisting of human  
20 angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fusions and fragments thereof, in the preparation of a

medicament for treating a human patient suffering from diabetic retinopathy, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the retina.

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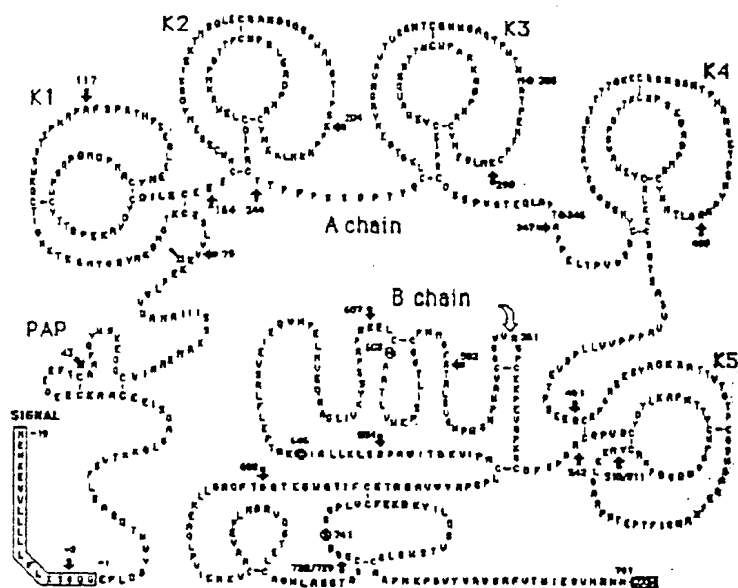


FIG. 1

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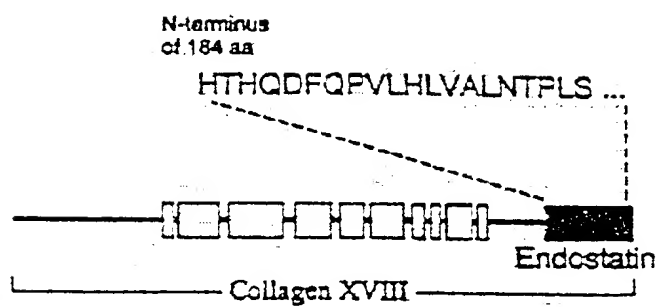


Fig. 2

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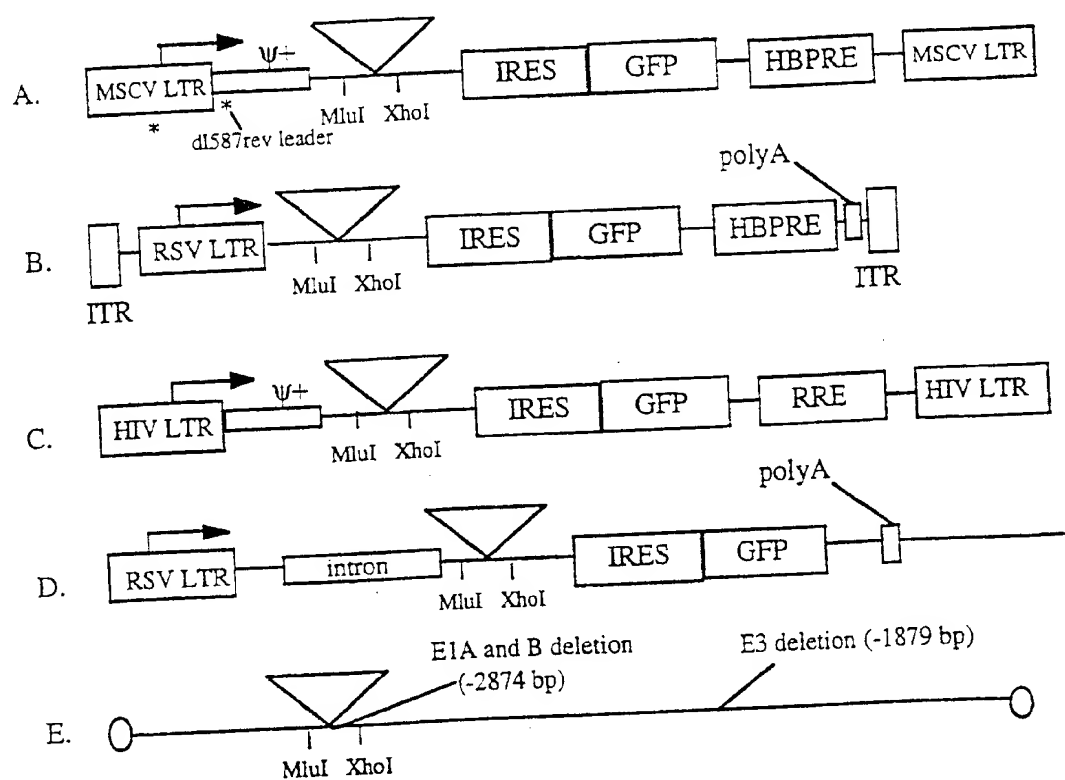


FIG. 3



1731





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[illegible]

end mouse endostatin coding sequence

Fig 6

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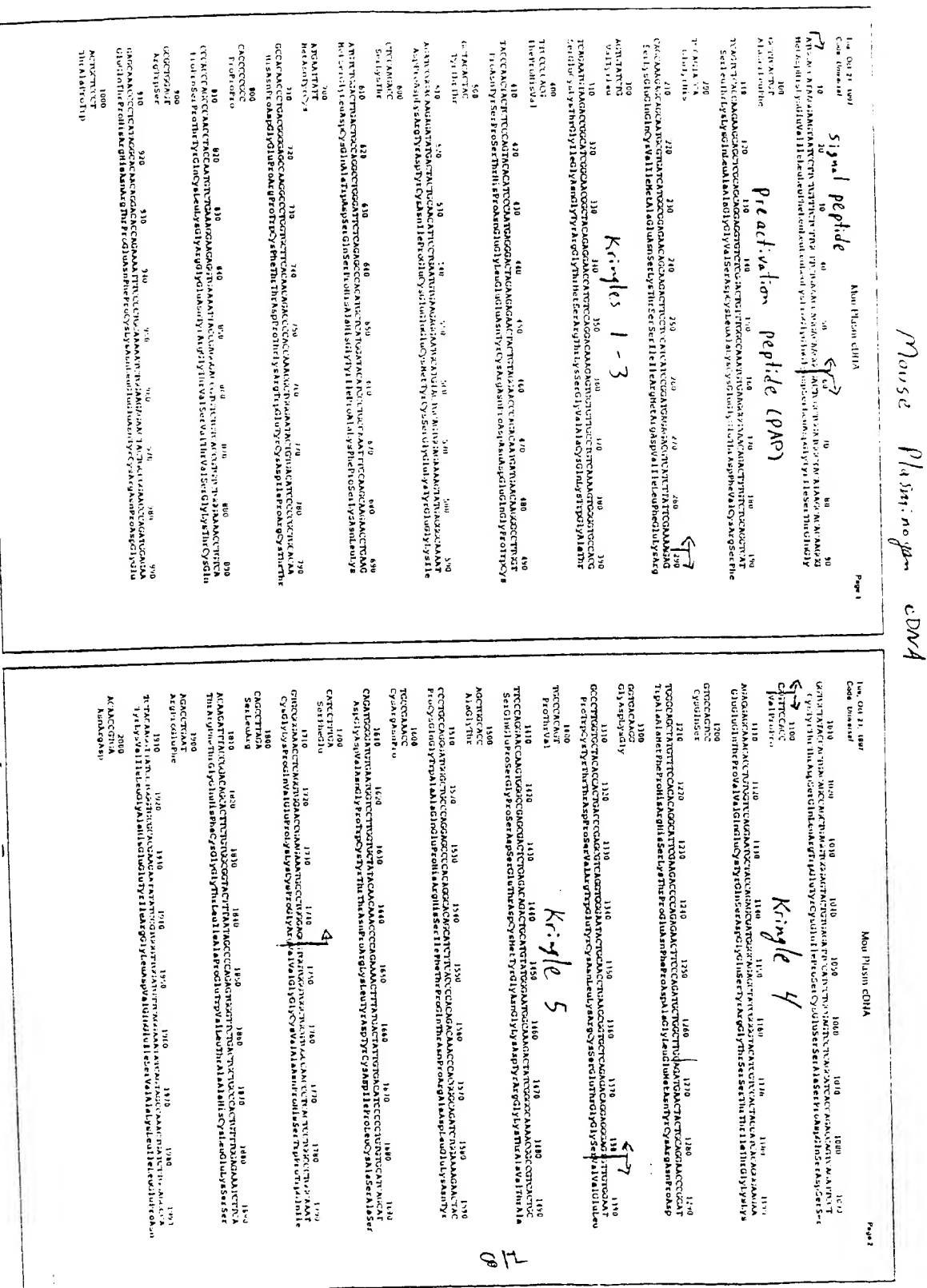


FIG. 7 (Sheet 1 of 2)

Thu, Oct 21, 1997  
Code: Universal

Mod Plasm cDNA

Page 3

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CCCGACAATA
ArgThrIle

1110      1120      1130      1140      1150      1160      1170      1180      1190
TCTTACATCACCGGCTGGGGAGAGACTCAAGGGACTTTCGGTGCCGGTCCGTCTCAAGGAGGCTCAGCTCCCTGTGATTGAGAACAAAGGTG
CysTyrIleThrGlyTrpGlyGluThrGlnGlyThrPheGlyAlaGlyArgLeuLysGluAlaGlnLeuProValIleGluAsnLysVal

1200
TCCAACCGCG
CysAsnArgVal

1210      1220      1230      1240      1250      1260      1270      1280      1290
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1300
GACCTCTGGT
ProLeuVal

1310      1320      1330      1340      1350      1360      1370      1380      1390
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1400
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ValSerArg

1410      1420      1430      1440      1450      1460      1470      1480      1490
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1500
GGGAGAGGGG
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1510      1520      1530      1540      1550      1560      1570      1580      1590
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1600
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TyrTyrCys

1610      1620      1630      1640      1650      1660      1670      1680      1690
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1700
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end plasminogen coding sequence.

## SEQUENCE LISTING

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<120> ANTI-ANGIOGENIC GENE THERAPY VECTORS AND  
THEIR USE IN TREATING ANGIOGENESIS-RELATED DISEASES

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ggt caa gga gag cct ctg gat gac tat gtg aat acc cag ggg gct tca	96
Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser	
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ctg ttc agt gtc act aag aag cag ctg gga gca gga agt ata gaa gaa	144
Leu Phe Ser Val Thr Lys Lys Gln Leu Gly Ala Gly Ser Ile Glu Glu	
35 40 45	
tgt gca gca aaa tgt gag gag gac gaa gaa ttc acc tgc agg gca ttc	192
Cys Ala Ala Lys Cys Glu Glu Asp Glu Glu Phe Thr Cys Arg Ala Phe	
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caa tat cac agt aaa gag caa caa tgt gtg ata atg gct gaa aac agg	240
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg	
65 70 75 80	
aag tcc tcc ata atc att agg atg aga gat gta gtt tta ttt gaa aag	288
Lys Ser Ser Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu Lys	
85 90 95	

aaa gtg tat ctc tca gag tgc aag act ggg aat gga aag aac tac aga	336
Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg	
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Gly Thr Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser	
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Ser Thr Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser	
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Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln	
145 150 155 160	
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Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys	
165 170 175	
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Asp Ile Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn	
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tat gac ggc aaa att tcc aag acc atg tct gga ctg gaa tgc cag gcc	624
Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala	
195 200 205	
tgg gac tct cag agc cca cac gct cat gga tac att cct tcc aaa ttt	672
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe	
210 215 220	
cca aac aag aac ctg aag aag aat tac tgt cgt aac ccc gat agg gag	720
Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu	
225 230 235 240	
ctg cgg cct tgg tgt ttc acc acc gac ccc aac aag cgc tgg gaa ctt	768
Leu Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu	
245 250 255	
tgc gac atc ccc cgc tgc aca aca cct cca cca tct tct ggt ccc acc	816
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr	
260 265 270	
tac cag tgt ctg aag gga aca ggt gaa aac tat cgc ggg aat gtg gct	864
Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala	
275 280 285	
gtt acc gtt tcc ggg cac acc tgt cag cac tgg agt gca cag acc cct	912
Val Thr Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro	
290 295 300	
cac aca cat aac agg aca cca gaa aac ttc ccc tgc aaa aat ttg gat	960
His Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp	
305 310 315 320	

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 Asp Ser Ser Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro  
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 Glu Leu Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser  
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 Tyr Arg Gly Thr Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser  
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tgg tca tct atg aca cca cac cgg cac cag aag acc cca gaa aac tac 1248  
 Trp Ser Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr  
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gtt act ggg acg cca tgc cag gac tgg gct gcc cag gag ccc cat aga 1536  
 Val Thr Gly Thr Pro Cys Gln Asp Trp Ala Ala Gln Glu Pro His Arg  
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cac agc att ttc act cca gag aca aat cca cgg gcg ggt ctg gaa aaa 1584  
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gcg gcc cct tca ttt gat tgt ggg aag cct caa gtg gag ccg aag aaa			1728
Ala Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys			
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Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro His Ser Trp			
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Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu			
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Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His			
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Gln Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu Val Ser Arg			
	645	650	655
ctg ttc ttg gag ccc aca cga aaa gat att gcc ttg cta aag cta agc			2016
Leu Phe Leu Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu Lys Leu Ser			
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Ser Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser			
	675	680	685
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Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly Trp			
	690	695	700
gga gaa acc caa ggt act ttt gga gct ggc ctt ctc aag gaa gcc cag			2160
Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys Glu Ala Gln			
	705	710	715
ctc cct gtg att gag aat aaa gtg tgc aat cgc tat gag ttt ctg aat			2208
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Gly Arg Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly			
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 770 775 780  
 gca cgc ccc aat aag cct ggt gtc tat gtt cgt gtt tca agg ttt gtt 2400  
 Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val  
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 Cys Ala Ala Lys Cys Glu Glu Asp Glu Glu Phe Thr Cys Arg Ala Phe  
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 Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg  
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 Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg  
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 Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys  
 165 170 175  
 Asp Ile Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn  
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 Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala  
 195 200 205  
 Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe  
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 Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu  
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Trp	Ser	Ser	Met	Thr	Pro	His	Arg	His	Gln	Lys	Thr	Pro	Glu	Asn	Tyr
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Lys	Gly	Pro	Trp	Cys	Phe	Thr	Thr	Asp	Pro	Ser	Val	Arg	Trp	Glu	Tyr
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Cys	Asn	Leu	Lys	Lys	Cys	Ser	Gly	Thr	Glu	Ala	Ser	Val	Val	Ala	Pro
450				455				460							
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Gln	Glu	Val	Asn	Leu	Glu	Pro	His	Val	Gln	Glu	Ile	Glu	Val	Ser	Arg
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660				665				670							
Ser	Pro	Ala	Val	Ile	Thr	Asp	Lys	Val	Ile	Pro	Ala	Cys	Leu	Pro	Ser
675				680				685							
Pro	Asn	Tyr	Val	Val	Ala	Asp	Arg	Thr	Glu	Cys	Phe	Ile	Thr	Gly	Trp
690				695				700							

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 Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Phe Leu Asn  
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 Gly Arg Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly  
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 755 760 765  
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 Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe  
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 Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser  
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 Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys  
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 Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg  
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Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn			
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Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln			
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ggg cct tgg tgc tac act aca gat cgg gac aag aga tat gac tac tgc			528
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165	170	175	
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Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys			
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Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala			
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Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr			
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Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser			
275	280	285	
gtc acc gtg tct ggg aaa acc tgt cag cgc tgg agt gag caa acc cct			912
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro			
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His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu			
305	310	315	320
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Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr			
325	330	335	
acc act gac agc cag ctg agg tgg gag tac tgt gag att cca tcc tgc			1056

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Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr	
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Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Ser Val Val Glu Leu	
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Pro Thr Val Ser Gln Glu Pro Ser Gly Pro Ser Asp Ser Glu Thr Asp	
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His Ser Ile Phe Thr Pro Gln Thr Asn Pro Arg Ala Asp Leu Glu Lys	
515 520 525	
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Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp Cys Tyr	
530 535 540	
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Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Ile Pro Leu Cys	
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660 665 670	
cta agc cgc cca gcc acc atc acg gat aaa gtc att cca cct tgt ctg	2064
Leu Ser Arg Pro Ala Thr Ile Thr Asp Lys Val Ile Pro Ala Cys Leu	
675 680 685	
cca tct cca aat tac atg gtt gct gac cgg aca ata tgt tac atc acc	2112
Pro Ser Pro Asn Tyr Met Val Ala Asp Arg Thr Ile Cys Tyr Ile Thr	
690 695 700	
ggc tgg gga gag act caa ggg act ttc ggt gcc ggt cgt ctg aag gag	2160
Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Arg Leu Lys Glu	
705 710 715 720	
gct cag ctg cct gtg att gag aac aag gtg tgc aac cgc gtc gag tat	2208
Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Val Glu Tyr	
725 730 735	
ctg aac aac aga gtc aaa tcc acg gag ctg tgt gcc ggg caa ctg gct	2256
Leu Asn Asn Arg Val Lys Ser Thr Glu Leu Cys Ala Gly Gln Leu Ala	
740 745 750	
ggt ggc gtc gac agc tgc caa ggc gac agt gga gga cct ctg gtt tgc	2304
Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys	
755 760 765	
ttc gag aag gac aag tac att tta caa gga gtc act tct tgg ggt ctt	2352
Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu	
770 775 780	

ggc tgt gct cgc ccc aat aag cct ggt gtc tac gtt cgt gtc tca cgg 2400  
 Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg  
 785 790 795 800  
 ttt gtt gat tgg att gaa agg gag atg agg aat aac tgactaggtg 2446  
 Phe Val Asp Trp Ile Glu Arg Glu Met Arg Asn Asn  
 805 810  
 gaaggccgag caaaacctct gcttactaaa gcttactgaa tatggggaga gggccttaggg 2506  
 tgtttgga aactgacagt aatcaaaactg ggacactaca ctgaaccaca gcttctgtc 2566  
 gccctcagc cctccctct tttttgtatt attgtgggta aaattttctt gtctgtggac 2626  
 ttctggattt tgtgacaata gaccatcact gctgtgacct ttgttgaaaa taaactcgat 2686  
 acttactttg 2696

<210> 4  
 <211> 812  
 <212> PRT  
 <213> Mus musculus

<400> 4  
 Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Lys Pro  
 1 5 10 15  
 Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser  
 20 25 30  
 Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp  
 35 40 45  
 Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe  
 50 55 60  
 Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser  
 65 70 75 80  
 Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys  
 85 90 95  
 Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg  
 100 105 110  
 Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly  
 115 120 125  
 Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn  
 130 135 140  
 Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln  
 145 150 155 160  
 Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys  
 165 170 175  
 Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys  
 180 185 190  
 Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala  
 195 200 205  
 Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe  
 210 215 220  
 Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu  
 225 230 235 240  
 Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr  
 245 250 255  
 Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr  
 260 265 270

Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser  
 275 280 285  
 Val Thr Val Ser Gly Lys Thr Dys Gln Arg Trp Ser Glu Gln Thr Pro  
 290 295 300  
 His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu  
 305 310 315 320  
 Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr  
 325 330 335  
 Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys  
 340 345 350  
 Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu  
 355 360 365  
 Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser  
 370 375 380  
 Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser  
 385 390 395 400  
 Trp Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe  
 405 410 415  
 Pro Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp  
 420 425 430  
 Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr  
 435 440 445  
 Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Ser Val Val Glu Leu  
 450 455 460  
 Pro Thr Val Ser Gln Glu Pro Ser Gly Pro Ser Asp Ser Glu Thr Asp  
 465 470 475 480  
 Cys Met Tyr Gly Asn Gly Lys Asp Tyr Arg Gly Lys Thr Ala Val Thr  
 485 490 495  
 Ala Ala Gly Thr Pro Cys Gln Gly Trp Ala Ala Gln Glu Pro His Arg  
 500 505 510  
 His Ser Ile Phe Thr Pro Gln Thr Asn Pro Arg Ala Asp Leu Glu Lys  
 515 520 525  
 Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp Cys Tyr  
 530 535 540  
 Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Ile Pro Leu Cys  
 545 550 555 560  
 Ala Ser Ala Ser Ser Phe Glu Cys Gly Lys Pro Gln Val Glu Pro Lys  
 565 570 575  
 Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Pro His Ser  
 580 585 590  
 Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg Phe Thr Gly Gln His Phe  
 595 600 605  
 Cys Gly Gly Thr Leu Ile Ala Pro Glu Trp Val Leu Thr Ala Ala His  
 610 615 620  
 Cys Leu Glu Lys Ser Ser Arg Pro Glu Phe Tyr Lys Val Ile Leu Gly  
 625 630 635 640  
 Ala His Glu Glu Tyr Ile Arg Gly Leu Asp Val Gln Glu Ile Ser Val  
 645 650 655  
 Ala Lys Leu Ile Leu Glu Pro Asn Asn Arg Asp Ile Ala Leu Leu Lys  
 660 665 670  
 Leu Ser Arg Pro Ala Thr Ile Thr Asp Lys Val Ile Pro Ala Cys Leu  
 675 680 685  
 Pro Ser Pro Asn Tyr Met Val Ala Asp Arg Thr Ile Cys Tyr Ile Thr  
 690 695 700  
 Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Arg Leu Lys Glu



705                      710                      715                      720  
 Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Val Glu Tyr  
                          725                      730                      735  
 Leu Asn Asn Arg Val Lys Ser Thr Glu Leu Cys Ala Gly Gln Leu Ala  
                          740                      745                      750  
 Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys  
                          755                      760                      765  
 Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu  
                          770                      775                      780  
 Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg  
 785                      790                      795                      800  
 Phe Val Asp Trp Ile Glu Arg Glu Met Arg Asn Asn  
                          805                      810

<210> 5  
 <211> 1083  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1)...(1083)

<400> 5  
 tat ctc tca gag tgc aag act ggg aat gga aag aac tac aga ggg acg                      48  
 Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg Gly Thr  
                          1                      5                      10                      15  
 atg tcc aaa aca aaa aat ggc atc acc tgt caa aaa tgg agt tcc act                      96  
 Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser Ser Thr  
                          20                      25                      30  
 tct ccc cac aga cct aga ttc tca cct gct aca cac ccc tca gag gga                      144  
 Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser Glu Gly  
                          35                      40                      45  
 ctg gag gag aac tac tgc agg aat cca gac aac gat cgg cag ggg ccc                      192  
 Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln Gly Pro  
                          50                      55                      60  
 tgg tgc tat act act gat cca gaa aag aga tat gac tac tgc gac att                      240  
 Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys Asp Ile  
                          65                      70                      75                      80  
 ctt gag tgt gaa gag gaa tgt atg cat tgc agt gga gaa aac tat gac                      288  
 Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn Tyr Asp  
                          85                      90                      95  
 ggc aaa att tcc aag acc atg tct gga ctg gaa tgc cag gcc tgg gac                      336  
 Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala Trp Asp  
                          100                      105                      110  
 tct cag agc cca cac gct cat gga tac att cct tcc aaa ttt cca aac                      384  
 Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe Pro Asn  
                          115                      120                      125

aag aac ctg aag aag aat tac tgt cgt aac ccc gat agg gac ctg cgg Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu Leu Arg 130 135 140	432
ccg tgg tgt ttc acc acc gac ccc aac aag cgc tgg gaa cct tgc gac Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu Cys Asp 145 150 155 160	480
atc ccc cgc tgc aca aca cct cca cca tct tct ggt ccc acc tac cag Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr Tyr Gln 165 170 175	528
tgt ctg aag gga aca ggt gaa aac tac cgc ggg aat gtg gct gct acc Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala Val Thr 180 185 190	576
gtt tcc ggg cac acc tgt cag cac tgg agt gca cag acc cct cac aca Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro His Thr 195 200 205	624
cat aac agg aca cca gaa aac ttc ccc tgc aaa aat ttg gat gaa aac His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp Glu Asn 210 215 220	672
tac tgc cgc aat cct gac gga aaa agg gcc cca tgg tgc cat aca acc Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His Thr Thr 225 230 235 240	720
aac agc caa gtg cgg tgg gag tac tgt aag ata ccg tcc tgt gac tcc Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys Asp Ser 245 250 255	768
tcc cca gta tcc acg gaa caa ttg gct ccc aca gca cca cct gag cta Ser Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro Glu Leu 260 265 270	816
acc cct gtg gtc cag gac tgc tac cat ggt gat gga cag agc tac cga Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser Tyr Arg 275 280 285	864
ggc aca tcc tcc acc acc acc aca gga aag aag tgt cag tct tgg tca Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser Trp Ser 290 295 300	912
tct atg aca cca cac cgg cac cag aag acc cca gaa aac tac cca aat Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr Pro Asn 305 310 315 320	960
gct ggc ctg aca atg aac tac tgc agg aat cca gat gcc gat aaa ggc Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp Lys Gly 325 330 335	1008
ccc tgg tgt ttt acc aca gac ccc agc gtc agg tgg gag tac tgc aac Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys Asn	1056

340 345 350  
 1083  
 ctg aaa aaa tgc tca gga aca gaa gcc  
 Leu Lys Lys Cys Ser Gly Thr Glu Ala  
 355 360

<210> 6  
 <211> 361  
 <212> PRT  
 <213> Homo sapiens

<400> 6  
 Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg Gly Thr  
 1 5 10 15  
 Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser Ser Thr  
 20 25 30  
 Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser Glu Gly  
 35 40 45  
 Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln Gly Pro  
 50 55 60  
 Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys Asp Ile  
 65 70 75 80  
 Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn Tyr Asp  
 85 90 95  
 Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala Trp Asp  
 100 105 110  
 Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe Pro Asn  
 115 120 125  
 Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu Leu Arg  
 130 135 140  
 Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu Cys Asp  
 145 150 155 160  
 Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr Tyr Gln  
 165 170 175  
 Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala Val Thr  
 180 185 190  
 Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro His Thr  
 195 200 205  
 His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp Glu Asn  
 210 215 220  
 Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His Thr Thr  
 225 230 235 240  
 Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys Asp Ser  
 245 250 255  
 Ser Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro Glu Leu  
 260 265 270  
 Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser Tyr Arg  
 275 280 285  
 Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser Trp Ser  
 290 295 300  
 Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr Pro Asn  
 305 310 315 320  
 Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp Lys Gly  
 325 330 335

Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys Asn  
 340 345 350  
 Leu Lys Lys Cys Ser Gly Thr Glu Ala  
 355 360

<210> 7  
 <211> 1086  
 <212> DNA  
 <213> Mus musculus

<220>  
 <221> CDS  
 <222> (1)...(1086)

<400> 7  
 gtg tat ctg tca gaa tgt aag acc ggc atc ggc aac gcc tac aga gga 48  
 Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg Gly  
 1 5 10 15  
 acc atg tcc agg aca aag agt ggt gtt gcc tgt caa aag tgg ggt gcc 96  
 Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly Ala  
 20 25 30  
 acg ttc ccc cac gta ccc aac tac tct ccc agt aca cat ccc aat gag 144  
 Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn Glu  
 35 40 45  
 gga cta gaa gag aac tac tgt agg aac cca gac aat gat gaa caa ggg 192  
 Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln Gly  
 50 55 60  
 cct tgg tgc tac act aca gat ccg gac aag aga tat gac tac tgc aac 240  
 Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys Asn  
 65 70 75 80  
 att cct gaa tgt gaa gag gaa tgc atg tac tgc agt gga gaa aag tat 288  
 Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys Tyr  
 85 90 95  
 gag ggc aaa atc tcc aag acc atg tct gga ctt gac tgc cag gcc tgg 336  
 Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala Trp  
 100 105 110  
 gat tct cag agc cca cat gct cat gga tac atc cct gcc aaa ttt cca 384  
 Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe Pro  
 115 120 125  
 agc aag aac ctg aag atg aat tat tgc cac aac cct gac ggg gag cca 432  
 Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu Pro  
 130 135 140  
 agg ccc tgg tgc ttc aca aca gac ccc acc aaa cgc tgg gaa tac tgt 480  
 Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr Cys  
 145 150 155 160

gac atc ccc cgc tgc aca aca ccc cgc ccc cca ccc agc cca acc tac 528  
 Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr Tyr  
 165 170 175

caa tgt ctg aaa gga aga ggt gaa aat tac cga ggg acc gtg tct gtc 576  
 Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser Val  
 180 185 190

acc gtg tct ggg aaa acc tgt cag cgc tgg agt gag caa acc cct cat 624  
 Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro His  
 195 200 205

agg cac aac agg aca cca gaa aat ttc ccc tgc aaa aat ctg gaa gag 672  
 Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu Glu  
 210 215 220

aac tac tgc cgg aac cca gat gga gaa act gct ccc tgg tgc tat acc 720  
 Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr Thr  
 225 230 235 240

act gac agc cag ctg agg tgg gag tac tgt gag att cca tcc tgc gag 768  
 Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys Glu  
 245 250 255

tcc tca gca tca cca gac cag tca gat tcc tca gtt cca cca gag gag 816  
 Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu Glu  
 260 265 270

caa aca cct gtg gtc cag gaa tgc tac cag agc gat ggg cag agc tat 864  
 Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser Tyr  
 275 280 285

cgg ggt aca tgc tcc act acc atc aca ggg aag aag tgc cag tcc tgg 912  
 Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser Trp  
 290 295 300

gca gct atg ttt cca cac agg cat tgc aag acc cca gag aac ttc cca 960  
 Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe Pro  
 305 310 315 320

gat gct ggc ttg gag atg aac tac tgc agg aac ccg gat ggt gac aag 1008  
 Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp Lys  
 325 330 335

ggc cct tgg tgc tac acc act gac ccg agc gtc agg tgg gaa tac tgc 1056  
 Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys  
 340 345 350

aac ctg aag cgg tgc tca gag aca gga ggg 1086  
 Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly  
 355 360

&lt;210&gt; 8

&lt;211&gt; 362

<211> PRT  
<211> Mus musculus

<400> 8  
Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg Gly  
1 5 10 15  
Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly Ala  
20 25 30  
Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn Glu  
35 40 45  
Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln Gly  
50 55 60  
Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys Asn  
65 70 75 80  
Ile Pro Glu Cys Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys Tyr  
85 90 95  
Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala Trp  
100 105 110  
Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe Pro  
115 120 125  
Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu Pro  
130 135 140  
Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr Cys  
145 150 155 160  
Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr Tyr  
165 170 175  
Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser Val  
180 185 190  
Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro His  
195 200 205  
Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu Glu  
210 215 220  
Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr Thr  
225 230 235 240  
Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys Glu  
245 250 255  
Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu Glu  
260 265 270  
Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser Tyr  
275 280 285  
Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser Trp  
290 295 300  
Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe Pro  
305 310 315 320  
Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp Lys  
325 330 335  
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys  
340 345 350  
Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly  
355 360

<210> 9  
<211> 552  
<212> DNA  
<213> Mus musculus

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)...(552)

<400> 9  
cat act cat cag gac ttt caq cca ggg ctc cac ctg gtg gca ctg aac 48  
His Thr His Gln Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn  
1 5 10 15  
acc ccc ctg tct gga ggc atg cgt ggt atc cgt gga gca gat ttc cag 96  
Thr Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln  
20 25 30  
tgc ttc cag caa gcc cga gcc gtg ggg ctg tcg ggc acc ttc cgg gct 144  
Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ser Gly Thr Phe Arg Ala  
35 40 45  
ttc ctg tcc tct agg ctg cag gat ctc tat agc atc gtg cgc cgt gct 192  
Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala  
50 55 60  
gac cgg ggg tct gtg ccc atc gtc aac ctg aag gac gag gtg cta tct 240  
Asp Arg Gly Ser Val Pro Ile Val Asn Leu Lys Asp Glu Val Leu Ser  
65 70 75 80  
ccc agc tgg gac tcc ctg ttt tct ggc tcc cag ggt caa gtg caa ccc 288  
Pro Ser Trp Asp Ser Leu Phe Ser Gly Ser Gln Gly Gln Val Gln Pro  
85 90 95  
ggg gcc cgc atc ttt tct ttt gac ggc aga gat gtc ctg aga cac cca 336  
Gly Ala Arg Ile Phe Ser Phe Asp Gly Arg Asp Val Leu Arg His Pro  
100 105 110  
gcc tgg ccg cag aag agc gta tgg cac ggc tcg gac ccc agt ggg cgg 384  
Ala Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Ser Gly Arg  
115 120 125  
agg ctg atg gag agt tac tgt gag aca tgg cga act gaa act act ggg 432  
Arg Leu Met Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr Thr Gly  
130 135 140  
gct aca ggt cag gcc tcc tcc ctg ctg tca ggc agg ctc ctg gaa cag 480  
Ala Thr Gly Gln Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu Glu Gln  
145 150 155 160  
aaa gct gcg agc tgc cac aac agc tac atc gtc ctg tgc att gag aat 528  
Lys Ala Ala Ser Cys His Asn Ser Tyr Ile Val Leu Cys Ile Glu Asn  
165 170 175  
agc ttc atg acc tct ttc tcc aaa 552  
Ser Phe Met Thr Ser Phe Ser Lys  
180

&lt;210&gt; 10

<111> 164  
 <112> PRT  
 <113> Mus musculus

<400> 10  
 His Thr His Gln Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn  
 1 5 10 15  
 Thr Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln  
 20 25 30  
 Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ser Gly Thr Phe Arg Ala  
 35 40 45  
 Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala  
 50 55 60  
 Asp Arg Gly Ser Val Pro Ile Val Asn Leu Lys Asp Glu Val Leu Ser  
 65 70 75 80  
 Pro Ser Trp Asp Ser Leu Phe Ser Gly Ser Gln Gly Gln Val Gln Pro  
 85 90 95  
 Gly Ala Arg Ile Phe Ser Phe Asp Gly Arg Asp Val Leu Arg His Pro  
 100 105 110  
 Ala Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Ser Gly Arg  
 115 120 125  
 Arg Leu Met Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr Thr Gly  
 130 135 140  
 Ala Thr Gly Gln Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu Glu Gln  
 145 150 155 160  
 Lys Ala Ala Ser Cys His Asn Ser Tyr Ile Val Leu Cys Ile Glu Asn  
 165 170 175  
 Ser Phe Met Thr Ser Phe Ser Lys  
 180

<210> 11  
 <211> 1414  
 <212> DNA  
 <213> Mus musculus

<220>  
 <221> CDS  
 <222> (1)...(1414)

<400> 11  
 atg gac cat aag gaa gta atc ctt ctg ttt ctc ttg ctt ctg aaa cca 48  
 Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Lys Pro  
 1 5 10 15  
 gga caa ggg gac tgg ctg gat ggc tac ata agc aca caa ggg gct tca 96  
 Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser  
 20 25 30  
 ctg ttc agt ctc acc aag aag cag ctc gca gca gga ggt gtc tgg gac 144  
 Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp  
 35 40 45  
 tgt ttg gcc aaa tgt gaa ggg gaa aca gac ttt gtc tgc agg tca ttc 192  
 Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe  
 50 55 60



cag tac cac agc aaa gag cag caa tgc gtg atc atg gcg gag aac agc 240  
 Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser  
 65 70 75 80

aag act tcc tcc atc atc egg atg aga gac gtc atc tta ttc gaa aag 288  
 Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys  
 85 90 95

aga gtg tat ctg tca gaa tgt aag acc gcc atc gcc aac gcc tac aga 336  
 Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg  
 100 105 110

gga acc atg tcc agg aca aag agt ggt gtt gcc tgt caa aag tgg ggt 384  
 Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly  
 115 120 125

gcc acg ttc ccc cac gta ccc aac tac tct ccc agt aca cat ccc aat 432  
 Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn  
 130 135 140

gag gga cta gaa gag aac tac tgt agg aac cca gac aat gat gaa caa 480  
 Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln  
 145 150 155 160

ggg cct tgg tgc tac act aca gat ccg gac aag aga tat gac tac tgc 528  
 Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys  
 165 170 175

aac att cct gaa tgt gaa gag gaa tgc atg tac tgc agt gga gaa aag 576  
 Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys  
 180 185 190

tat gag ggc aaa atc tcc aag acc atg tct gga ctt gac tgc cag gcc 624  
 Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala  
 195 200 205

tgg gat tct cag agc cca cat gct cat gga tac atc cct gcc aaa ttt 672  
 Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe  
 210 215 220

cca agc aag aac ctg aag atg aat tat tgc cac aac cct gac ggg gag 720  
 Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu  
 225 230 235 240

cca agg ccc tgg tgc ttc aca aca gac ccc acc aaa cgc tgg gaa tac 768  
 Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr  
 245 250 255

tgt gac atc ccc cgc tgc aca aca ccc ccg ccc cca ccc agc cca acc 816  
 Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Ser Pro Thr  
 260 265 270

tac caa tgt ctg aaa gga aga ggt gaa aat tac cga ggg acc gtg tct 864  
 Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser

275	280	285	
gtc acc gtg tct ggg aaa acc tgt cag cgc tgg agt gag caa acc cct			912
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro			
290	295	300	
cat agg cac aac agg aca cca gaa aat ttc ccc tgc aaa aat ctg gaa			960
His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu			
305	310	315	320
gag aac tac tgc cgg aac cca gat gga gaa act gct ccc tgg tgc tat			1008
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr			
325	330	335	
acc act gac agc cag ctg agg tgg gag tac tgt gag att cca tcc tgc			1056
Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys			
340	345	350	
gag tcc tca gca tca cca gac cag tca gat tcc tca gtt cca cca gag			1104
Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu			
355	360	365	
gag caa aca cct gtg gtc cag gaa tgc tac cag agc gat ggg cag agc			1152
Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser			
370	375	380	
tat cgg ggt aca tgc tcc act acc atc aca ggg aag aag tgc cag tcc			1200
Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser			
385	390	395	400
tgg gca gct atg ttt cca cac agg cat tgg aag acc cca gag aac ttc			1248
Trp Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe			
405	410	415	
cca gat gct ggc ttg gag atg aac tac tgc agg aac ccg gat ggt gac			1296
Pro Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp			
420	425	430	
aag ggc cct tgg tgc tac acc act gac ccg agc gtc agg tgg gaa tac			1344
Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr			
435	440	445	
tgc aac ctg aag cgg tgc tca gag aca gga ggg aat tca gac tac aag			1392
Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Asn Ser Asp Tyr Lys			
450	455	460	
gac gac gat gac aag taa taa c			1414
Asp Asp Asp Asp Lys * *			
465			

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 <212> PRT  
 <213> Mus musculus

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 Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp  
 35 40 45  
 Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe  
 50 55 60  
 Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser  
 65 70 75 80  
 Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys  
 85 90 95  
 Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg  
 100 105 110  
 Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly  
 115 120 125  
 Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn  
 130 135 140  
 Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln  
 145 150 155 160  
 Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys  
 165 170 175  
 Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys  
 180 185 190  
 Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala  
 195 200 205  
 Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe  
 210 215 220  
 Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu  
 225 230 235 240  
 Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr  
 245 250 255  
 Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr  
 260 265 270  
 Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser  
 275 280 285  
 Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro  
 290 295 300  
 His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu  
 305 310 315 320  
 Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr  
 325 330 335  
 Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys  
 340 345 350  
 Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu  
 355 360 365  
 Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser  
 370 375 380  
 Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser  
 385 390 395 400  
 Trp Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe  
 405 410 415  
 Pro Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp  
 420 425 430

Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr  
 435 440 445  
 Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Asn Ser Asp Tyr Lys  
 450 455 460  
 Asp Asp Asp Asp Lys  
 465

<210> 13  
 <211> 661  
 <212> DNA  
 <213> Mus musculus

<220>  
 <221> CDS  
 <222> (1)...(661)

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 1 5 10 15  
 gga caa ggg gac tgg cta gat ctt gac tac aag gac gac gat gac aag 96  
 Gly Gln Gly Asp Ser Leu Asp Leu Asp Tyr Lys Asp Asp Asp Asp Lys  
 20 25 30  
 ctt gct cat act cat cag gac ttt cag cca gtg ctc cac ctg gtg gca 144  
 Leu Ala His Thr His Gln Asp Phe Gln Pro Val Leu His Leu Val Ala  
 35 40 45  
 ctg aac acc ccc ctg tct gga ggc atg cgt ggt atc cgt gga gca gat 192  
 Leu Asn Thr Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp  
 50 55 60  
 ttc cag tgc ttc cag caa gcc cga gcc gtg ggg ctg tgg ggc acc ttc 240  
 Phe Gln Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ser Gly Thr Phe  
 65 70 75 80  
 cgg gct ttc ctg tcc tct agg ctg cag gat ctc tat agc atc gtg cgc 288  
 Arg Ala Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg  
 85 90 95  
 cgt gct gac cgg ggg tct gtg ccc atc gtc aac ctg aag gac gag gtg 336  
 Arg Ala Asp Arg Gly Ser Val Pro Ile Val Asn Leu Lys Asp Glu Val  
 100 105 110  
 cta tct ccc agc tgg gac tcc ctg ttt tct ggc tcc cag ggt caa gtg 384  
 Leu Ser Pro Ser Trp Asp Ser Leu Phe Ser Gly Ser Gln Gly Gln Val  
 115 120 125  
 caa ccc ggg gcc cgc atc ttt tct ttt gac ggc aga gat gtc ctg aga 432  
 Gln Pro Gly Ala Arg Ile Phe Ser Phe Asp Gly Arg Asp Val Leu Arg  
 130 135 140  
 cac cca gcc tgg ccg cag aag agc gta tgg cac gcc tgg gac ccc agt 480  
 His Pro Ala Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Ser

145                      150                      155                      160  
 ggg cgg agg ctg atg gag agt tac tgt gag aca tgg cga act gaa act      528  
 Gly Arg Arg Leu Met Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr  
                          165                      170                      175  
 act ggg gct aca ggt cag gcc tcc tcc ctg ctg tca ggc agg ctc ctg      576  
 Thr Gly Ala Thr Gly Gln Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu  
                          180                      185                      190  
 gaa cag aaa gct gcg agc tgc cac aac agc tac atc gtc ctg tgc att      624  
 Glu Gln Lys Ala Ala Ser Cys His Asn Ser Tyr Ile Val Leu Cys Ile  
                          195                      200                      205  
 gag aat agc ttc atg acc tct ttc tcc aaa taa taa c      661  
 Glu Asn Ser Phe Met Thr Ser Phe Ser Lys \* \*  
                          210                      215

<210> 14  
 <211> 218  
 <212> PRT  
 <213> Mus musculus

<400> 14  
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                          20                      25                      30  
 Leu Ala His Thr His Gln Asp Phe Gln Pro Val Leu His Leu Val Ala  
                          35                      40                      45  
 Leu Asn Thr Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp  
                          50                      55                      60  
 Phe Gln Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ser Gly Thr Phe  
                          65                      70                      75                      80  
 Arg Ala Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg  
                          85                      90                      95  
 Arg Ala Asp Arg Gly Ser Val Pro Ile Val Asn Leu Lys Asp Glu Val  
                          100                      105                      110  
 Leu Ser Pro Ser Trp Asp Ser Leu Phe Ser Gly Ser Gln Gly Gln Val  
                          115                      120                      125  
 Gln Pro Gly Ala Arg Ile Phe Ser Phe Asp Gly Arg Asp Val Leu Arg  
                          130                      135                      140  
 His Pro Ala Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Ser  
                          145                      150                      155                      160  
 Gly Arg Arg Leu Met Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr  
                          165                      170                      175  
 Thr Gly Ala Thr Gly Gln Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu  
                          180                      185                      190  
 Glu Gln Lys Ala Ala Ser Cys His Asn Ser Tyr Ile Val Leu Cys Ile  
                          195                      200                      205  
 Glu Asn Ser Phe Met Thr Ser Phe Ser Lys  
                          210                      215

<210> 15

<211> 1747  
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<220>  
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 <222> (1)...(1747)

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 1 5 10 15

gga caa ggg gac tgg ctg gat ggc tac ata agc ata caa ggg gct tca 96  
 Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser  
 20 25 30

ctg ttc agt ctc acc aag aag cag ctc gca gca gga ggt gtc tgg gac 144  
 Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp  
 35 40 45

tgt ttg gcc aaa tgt gaa ggg gaa aca gac ttt gtc tgc agg tca ttc 192  
 Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe  
 50 55 60

cag tac cac agc aaa gag cag caa tgc gtg atc atg gcg gag aac agc 240  
 Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser  
 65 70 75 80

aag act tcc tcc atc atc cgg atg aga gac gtc atc tta ttc gaa aag 288  
 Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys  
 85 90 95

aga gtg tat ctg tca gaa tgt aag acc ggc atc ggc aac ggc tac aga 336  
 Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg  
 100 105 110

gga acc atg tcc agg aca aag agt ggt gtt gcc tgt caa aag tgg ggt 384  
 Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly  
 115 120 125

gcc acg ttc ccc cac gta ccc aac tac tct ccc agt aca cat ccc aat 432  
 Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn  
 130 135 140

gag gga cta gaa gag aac tac tgt agg aac cca gac aat gat gaa caa 480  
 Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln  
 145 150 155 160

ggg cct tgg tgc tac act aca gat ccg gac aag aga tat gac tac tgc 528  
 Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys  
 165 170 175

aac att cct gaa tgt gaa gag gaa tgc atg tac tgc agt gga gaa aag 576  
 Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys

180	185	190	
tat gag ggc aaa atc tcc aag acc atg tct gga ctt gac tgc cag gcc			624
Tyr Glu Gly Lys Ile Ser Lys	Thr Met Ser Gly Leu Asp Cys Gln Ala		
195	200	205	
tgg gat tct cag agc cca cat gct cat gga tac atc cct gcc aaa ttt			672
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe			
210	215	220	
cca agc aag aac ctg aag atg aat tat tgc cac aac cct gac ggg gag			720
Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu			
225	230	235	240
cca agg ccc tgg tgc ttc aca aca gac ccc acc aaa cgc tgg gaa tac			768
Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr			
245	250	255	
tgt gac atc ccc cgc tgc aca aca ccc ccg ccc cca ccc agc cca acc			816
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Ser Pro Thr			
260	265	270	
tac caa tgt ctg aaa gga aga ggt gaa aat tac cga ggg acc gtg tct			864
Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser			
275	280	285	
gtc acc gtg tct ggg aaa acc tgt cag cgc tgg agt gag caa acc cct			912
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro			
290	295	300	
cat agg cac aac agg aca cca gaa aat ttc ccc tgc aaa aat ctg gaa			960
His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu			
305	310	315	320
gag aac tac tgc cgg aac cca gat gga gaa act gct ccc tgg tgc tat			1008
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr			
325	330	335	
acc act gac agc cag ctg agg tgg gag tac tgt gag att cca tcc tgc			1056
Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys			
340	345	350	
gag tcc tca gca tca cca gac cag tca gat tcc tca gtt cca cca gag			1104
Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu			
355	360	365	
gag caa aca cct gtg gga ggg aat tgc ggc ggt gga tca ggt ggc gga			1152
Glu Gln Thr Pro Val Gly Gly Asn Cys Gly Gly Gly Ser Gly Gly Gly			
370	375	380	
gat ctt gac tac aag gac gac gat gac aag ctt gct cat act cat cag			1200
Asp Leu Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala His Thr His Gln			
385	390	395	400
gac ttt cag cca gtg etc cac ctg gtg gca ctg aac acc ccc ctg tct			1248

Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn Thr Pro Leu Ser  
 405 410 415  
 gga ggc atg cgt ggt atc cgt gga gca gat ttc cag tgc ttc cag caa 1296  
 Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln Cys Phe Gln Gln  
 420 425 430  
 gcc cga gcc gtg ggg ctg tgg ggc acc ttc cgg gct ttc ctg tcc tct 1344  
 Ala Arg Ala Val Gly Leu Ser Gly Thr Phe Arg Ala Phe Leu Ser Ser  
 435 440 445  
 agg ctg cag gat ctg tat agc atc gtg cgc cgt gct gac cgg ggg tct 1392  
 Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala Asp Arg Gly Ser  
 450 455 460  
 gtg ccc atc gtc aac ctg aag gac gag gtg cta tct ccc acc tgg gac 1440  
 Val Pro Ile Val Asn Leu Lys Asp Glu Val Leu Ser Pro Ser Trp Asp  
 465 470 475 480  
 tcc ctg ttt tct ggc tcc cag ggt caa gtg caa ccc ggg gcc cgc atc 1488  
 Ser Leu Phe Ser Gly Ser Gln Gly Gln Val Gln Pro Gly Ala Arg Ile  
 485 490 495  
 ttt tct ttt gac ggc aga gat gtc ctg aga cac cca gcc tgg ccg cag 1536  
 Phe Ser Phe Asp Gly Arg Asp Val Leu Arg His Pro Ala Trp Pro Gln  
 500 505 510  
 aag agc gta tgg cac ggc tgg gac ccc agt ggg cgg agg ctg atg gag 1584  
 Lys Ser Val Trp His Gly Ser Asp Pro Ser Gly Arg Arg Leu Met Glu  
 515 520 525  
 agt tac tgt gag aca tgg cga act gaa act act ggg gct aca ggt cag 1632  
 Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr Thr Gly Ala Thr Gly Gln  
 530 535 540  
 gcc tcc tcc ctg ctg tca ggc agg ctg ctg gaa cag aaa gct gcg agc 1680  
 Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu Glu Gln Lys Ala Ala Ser  
 545 550 555 560  
 tgc cac aac agc tac atc gtc ctg tgc att gag aat agc ttc atg acc 1728  
 Cys His Asn Ser Tyr Ile Val Leu Cys Ile Glu Asn Ser Phe Met Thr  
 565 570 575  
 tct ttc tcc aaa taa taa c 1747  
 Ser Phe Ser Lys \* \*  
 580

<210> 16  
 <211> 580  
 <212> PRT  
 <213> Mus musculus

<400> 16  
 Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Lys Pro



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 20 25 30  
 Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp  
 35 40 45  
 Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe  
 50 55 60  
 Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser  
 65 70 75 80  
 Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys  
 85 90 95  
 Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg  
 100 105 110  
 Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly  
 115 120 125  
 Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn  
 130 135 140  
 Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln  
 145 150 155 160  
 Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys  
 165 170 175  
 Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys  
 180 185 190  
 Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala  
 195 200 205  
 Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe  
 210 215 220  
 Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu  
 225 230 235 240  
 Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr  
 245 250 255  
 Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Ser Pro Thr  
 260 265 270  
 Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser  
 275 280 285  
 Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro  
 290 295 300  
 His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu  
 305 310 315 320  
 Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr  
 325 330 335  
 Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys  
 340 345 350  
 Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu  
 355 360 365  
 Glu Gln Thr Pro Val Gly Gly Asn Cys Gly Gly Gly Ser Gly Gly Gly  
 370 375 380  
 Asp Leu Asp Tyr Lys Asp Asp Asp Lys Leu Ala His Thr His Gln  
 385 390 395 400  
 Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn Thr Pro Leu Ser  
 405 410 415  
 Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln Cys Phe Gln Gln  
 420 425 430  
 Ala Arg Ala Val Gly Leu Ser Gly Thr Phe Arg Ala Phe Leu Ser Ser  
 435 440 445

Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala Asp Arg Gly Ser  
 450 455 460  
 Val Pro Ile Val Asn Leu Lys Asp Gln Val Leu Ser Pro Ser Trp Asp  
 465 470 475 480  
 Ser Leu Phe Ser Gly Ser Gln Gly Gln Val Gln Pro Gly Ala Arg Ile  
 485 490 495  
 Phe Ser Phe Asp Gly Arg Asp Val Leu Arg His Pro Ala Trp Pro Gln  
 500 505 510  
 Lys Ser Val Trp His Gly Ser Asp Pro Ser Gly Arg Arg Leu Met Glu  
 515 520 525  
 Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr Thr Gly Ala Thr Gly Gln  
 530 535 540  
 Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu Glu Gln Lys Ala Ala Ser  
 545 550 555 560  
 Cys His Asn Ser Tyr Ile Val Leu Cys Ile Glu Asn Ser Phe Met Thr  
 565 570 575  
 Ser Phe Ser Lys  
 580

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 <211> 549  
 <212> DNA  
 <213> Homo sapiens

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 gggggcatgc ggggcacccg cggggccgac ttccagtgtc tccagcagc gccggccgtg 120  
 gggctggcgg gcacattccg cgccttcccg tctcgcgcgc tgcaggacct gtacagcatc 180  
 gtgcgcctg cgcacgcgc agcctgccc atcgtaacc tcaaggacga gctgctgttt 240  
 cccagctggg aggtctgtt ctcaggctct gagggccgc tgaagcccg ggcacgcac 300  
 ttctccttg acggcaagga cgtcctgagg caccaccct ggccccagaa gagcgtgtgg 360  
 catggctcgg accccaacgg ggcaggctg accgagagct actgtgagac gtggcggacg 420  
 gaggtccct cggccacggg ccaggcctcc tcgctgctgg cgggcaggct cctggggcag 480  
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 gcctccaag 549

<210> 18  
 <211> 183  
 <212> PRT  
 <213> Homo sapiens

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 His Ser His Arg Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn  
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 Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ala Gly Thr Phe Arg Ala  
 35 40 45  
 Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala  
 50 55 60  
 Asp Arg Ala Ala Val Pro Ile Val Asn Leu Lys Asp Glu Leu Leu Phe  
 65 70 75 80  
 Pro Ser Trp Glu Ala Leu Phe Ser Gly Ser Glu Gly Pro Leu Lys Pro  
 85 90 95  
 Gly Ala Arg Ile Phe Ser Phe Asp Gly Lys Asp Val Leu Arg His Pro

100 105 110  
 Thr Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Asn Gly Arg  
 115 120 125  
 Arg Leu Thr Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Ala Pro Ser  
 130 135 140  
 Ala Thr Gly Gln Ala Ser Ser Leu Leu Gly Gly Arg Leu Leu Gly Gln  
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/24950

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A01N 63/00, 43/04; C12N 15/00; C07H 21/02

US CL : 424/93.1; 435/320.1; 514/44; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.1; 435/320.1; 514/44; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,792,845 A (O'REILLY et al.) 11 August 1998 (11.08.98), col. 4, lines 32-68, col. 5, lines 1-2, 51-68, col. 6, lines 1-8.	1-30, 33
Y	WO 97/23500 A1 (THE CHILDREN'S MEDICAL CENTER CORPORATION) 03 July 1997 (03.07.97), page 41, lines 3-33, page 42, lines 1-27.	4
X,P	WO 98/49321 A2 (RHONE-POULENC RORER) 05 November 1998 (05.11.98), page 44, 6-11, 25-33, page 45, lines 12-13, 29-35.	1, 5, 18, 20, 31
Y,P		2-4, 6-17, 19, 21-30, 32, 33

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

04 FEBRUARY 1999

Date of mailing of the international search report

08 MAR 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

SHIN-LIN CHEN

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No  
PCT US98 24950

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	WO 97/15666 A (THE CHILDREN'S MEDICAL CENTER CORPORATION) 01 May 1997 (01.05.97), page 20, lines 16-35, page 21, page 22, lines 1-16. page 59, lines 5-35, page 60, page 61, 1-6.	1-30, 33
Y	TANAKA, T. et al. Retroviral and adenoviral mediated transduction of angiostatin cDNA inhibits angiogenesis and tumor growth. Proceedings of the American Association for Cancer Research. March 1997 (03.97). Vol 38. page 264.	1-33
Y	WO 96/35774 A2 (THE CHILDREN'S MEDICAL CENTER CORPORATION) 14 November 1996 (14.11.96), page lines 33-36, pages 22-25, page 26, lines 1-33. pages 144-148.	1-30, 33
Y	WO 97/41824 A2(ABBOTT LABORATORIES) 13 November 1997 (13.11.97), page 5, lines 13-38, page 6, 1-18, page 60, lines 15-38, pages 61-62, page 63, lines 1-33.	1-30, 33
Y	WO 95/29242 A1 (THE CHILDREN'S MEDICAL CENTER CORPORATION) 02 November 1995 (02.11.95), page 21, lines 19-35, pages 22-27, page 28, lines 1-4. page 87, lines 4-35, page 88, page 89, lines 1-14.	1-30, 33
Y	O'REILLY, et al. Angiostatin induces and sustains dormancy of human primary tumors in mice. Nature Medicine, June 1996 (06.96), Vol. 2, No. 6, pages 689-692, especially pages 689-690.	1-30, 33
Y	O'REILLY, et al. Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth. Cell, 24 January 1997 (24.01.97), Vol. 88, pages 277-285, especially pages 279-280, 282.	1-30, 33

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/24950

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, WPIDS, MEDLINE, CAPLUS, BIOSIS, EMBASE

search terms: angiostatin, plasminogen, endostatin, collagen(w) XVIII, inhibit?(5a)tumor(5a)growth, tumor(5a)regress?,  
diabet?(p)retinopathy, plasmid, viral(5a) vector.

